Surveillance, Control, and Management of Resistant Gram-Negative Bacteria in Community and Healthcare Settings

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- **Epidemiology of ESBL-producing Escherichia coli from repeated prevalence studies over 11 years in a long-term-care facility**
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- **Nation-wide survey of screening practices to detect carriers of multi-drug resistant organisms upon admission to Swiss healthcare institutions**
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- **First reported nosocomial outbreak of NDM-1 producing Escherichia coli in Switzerland**
    - *Contribution of the candidate: epidemiological investigation, data collection, writing of the abstract*
- **An interventional quasi-experimental study to evaluate the impact of a rapid screening strategy in improving nosocomial Extended-Spectrum-Beta-Lactamase Producing Enterobacterales and Carbapenemases Producing Enterobacterales control in critically ill patients**
    - *Contribution of the candidate: elaboration of the study design & protocol, data collection, data analysis, writing of the manuscript.*

I certify that this thesis and the research behind were conducted or co-conducted by myself, and any external intellectual ideas or concept were entirely acknowledged using standard referencing practices.

Romain Martischang

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"In the long history of humankind (and animal kind, too) those who learned to collaborate and improvise most effectively have prevailed."— Charles Darwin

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Résumé

Cette thèse explore les caractéristiques épidémiologiques et les stratégies de contrôle des Entérobactéries productrices de Beta-Lactamases à Spectre Élargi (EP-BLSE) ou de Carbapénémases (EPC), afin de mieux comprendre et limiter leur diffusion. Premièrement, cette diffusion est expliquée par une dynamique de transmission complexe impliquant différents réservoirs communicants et variant en fonction de facteurs propres à l'espèce, au patient, au soin, et au milieu de soin. En particulier, les établissements de soins à long terme et le domicile restent des milieux sous-étudiés requérants une attention particulière pour mieux comprendre l'épidémiologie moléculaire de certains clones à risque. Deuxièmement, la surveillance et les mesures de contrôle des EP-BLSE et EPC sont hautement hétérogènes parmi et entre les pays, avec une absence de consensus définissant les candidats appropriés pour un dépistage à l'admission, ainsi que les méthodes diagnostiques inclues dans les politiques de dépistage. Cette variation entrave non seulement un contrôle adéquat des bactéries résistantes à l'échelle institutionnelle, mais aussi un contrôle des importations et des transmissions parmi les établissements de soins à l'échelle nationale. Cette thèse essaye d'améliorer notre compréhension de la dynamique de transmission et des tendances temporelles des *E.coli* et *K.pneumoniae* producteurs de BLSE parmi les établissements de soins à long terme et les domiciles, mais aussi d'améliorer les politiques de dépistage existantes pour les bactéries Gram-négatives résistantes.

Dans une première partie, une enquête de prévalence répétée dans un établissement de soins à long cours a observé une augmentation nette de EP-BLSE ainsi qu'une fluctuation clonale des ST131H30. Malgré un court suivi, une absence d'effet rebond suite à l'arrêt institutionnel des mesures contact ciblant les *E.coli* producteurs de BLSE (EC-BLSE) en 2019 est notée, soutenant les recommandations actuelles pour le

contrôle des EC-BLSE. La découverte fortuite de l'expansion clonale d'un sous-clone ST131H89 atypique associé avec de multiples épidémies prolongées et silencieuses, ainsi que la diffusion régionale parmi différents réservoirs humains et environnementaux dans l'Ouest de la Suisse requiert une surveillance détaillée.
Une revue systématique et étude de cohorte multicentrique prospective ont évalué la dynamique de transmission des EP-BLSE à domicile, et ont confirmé des taux significatifs d'acquisition et de transmission entre les habitants du domicile, particulièrement dans les premières semaines suivant le retour à domicile des patients index manquants d'autonomie. Des taux de transmission différents ont été observés entre *E.coli* et *K.pneumoniae*, renforçant l'évidence existante en milieu hospitalier.

Dans une deuxième partie, une enquête à l'échelle nationale a observé des pratiques de dépistage à l'admission adéquates pour les bactéries multirésistantes, mais parfois déficientes et hétérogènes pour certaines bactéries, facteurs de risques, et sites de prélèvements. A noter que les établissements avec une déficience dans le dépistage des VRE se trouvaient majoritairement en Suisse de l'Est, coïncidant avec une large épidémie de VRE impliquant de nombreux hôpitaux. Ces résultats soulignent le besoin de standards harmonisés et accessibles définissant les stratégies de dépistage pour les bactéries Gram-Négatives multi-résistantes parmi les établissements de santé Suisses. Un suivi de cette enquête pourrait être assuré par de futures études pour évaluer l'impact des standards susmentionnés, et possiblement pour investiguer un lien avec les tendances épidémiologiques locales.
Une investigation d'épidémie a révélé d'importants bénéfices secondaires du dépistage universel hebdomadaire, facilitant la détection précoce d'une épidémie institutionnelle et accélérant l'implémentation des mesures de contrôle. Finalement, une étude interventionnelle quasi-expérimentale a comparé le test LAMP avec les cultures phénotypiques pour accélérer l'implémentation des mesures de contrôle. Cette étude a

observé des performances diagnostiques sous-optimales du test LAMP pour les EP-BLSE et CPE lorsque celui-ci était directement appliqué sur l'échantillon. Cette étude conclue qu'en l'absence de programme de « diagnostic stewardship », le LAMP n'apporte aucun bénéfice dans un milieu à faible endémicité, ni pour arrêter les mesures contact non nécessaires parmi les patients aux soins intensifs, ni pour implémenter les mesures contact parmi les nouveaux cas détectés.

Abstract

This thesis explores epidemiological characteristics and infection control strategies of Enterobacterales producing Extended-Spectrum beta-lactamases (ESBL-PE) or carbapenemases (CPE) to better comprehend and limit their spread. First, this spread is explained by complex transmission dynamics among intersecting reservoirs, differing among species, patients, and care settings. In particular, long-term care facilities and households remain understudied settings warranting further monitoring and research to comprehend the molecular epidemiology of clones at risk. Second, surveillance and infection control measures of ESBL-PE and CPE are highly heterogeneous within and between countries, with no consensus defining the best candidates for admission screening and diagnostic methods included in screening policies. This variation hinders adequate nosocomial multidrug-resistant organism (MDRO) control at the institutional level, but also the control of importation events and inter-facility transmissions at the national level. This thesis further aimed to better understand the transmission dynamics and temporal trends of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* in understudied settings, and to improve screening strategies for nosocomial control of MDR-Gram-negative bacteria.

In the first section, repeated cross-sectional surveys in a university-affiliated long-term care facility observed an increasing prevalence of ESBL-EC, and a clonal fluctuation of ST131H30 from 2010 to 2020. Despite a relatively short follow-up period, the absence of a rebound effect following the discontinuation of contact precautions for ESBL-EC in 2019 supported the most recent guidelines for ESBL-PE control. The fortuitous detection of the clonal expansion of an atypical ST131H89 subclone associated with multiple silent and prolonged outbreaks, and its regional spread among different reservoirs from Western Switzerland warrants further monitoring. A systematic review and a multicentric

prospective cohort study assessing ESBL-PE transmission dynamics in household settings confirmed a significant acquisition and transmission rate among household members, especially early after discharge of index cases with impaired autonomy. Different transmission rates were observed between *E.coli* and *K.pneumoniae*, supporting available evidence from healthcare settings.

In the second section, a nation-wide survey of Swiss hospitals observed adequate MDRO admission screening practices, but highlighted the heterogeneity of risk factors and body sites used in screening strategies, and an epidemiological gap for vancomycin-resistant enterococci (VRE), *Acinetobacter baumanii*, and *Pseudomonas aeruginosa*. To note, deficient admission screening practices for VRE unveiled by spatial analysis in Eastern Switzerland coincided with a large multi-institution VRE outbreak. These findings highlighted the need for harmonized and accessible standards defining screening strategies targeting resistant Gram-negative bacteria among Swiss healthcare institutions. Future follow-up studies are warranted to evaluate the impact of such standards, and possibly to link current screening practices with regional epidemiological trends. An outbreak investigation revealed important side benefits from universal regular screening to facilitate early detection of a small institutional cluster of highly resistant Gram-negative bacteria and to accelerate infection control measures. An interventional quasi-experimental study compared a LAMP (Loop-Mediated Isothermal Amplification) assay against standard phenotypic cultures to accelerate the implementation of infection control measures. This study observed a suboptimal diagnostic accuracy of LAMP for ESBL-PE and CPE detection when directly performed on rectal swabs. This study also observed that under real-life conditions, and without proper diagnostic stewardship, there was no benefit of LAMP in a low-endemicity setting, neither for discontinuing unnecessary CP among critically ill patients screened at admission, nor for implementing CP among newly positive patients.

Table of Contents

Abbreviation list

3GC: Third-Generation Cephalosporin

3GCR: Third-Generation Cephalosporin resistant

AMR: Antimicrobial Resistance

AMS: Antimicrobial Stewardship

CDC: Center for Disease Control and Prevention

CPE: Carbapenemase-producing Enterobacterales

ECDC: European Centre for Disease Prevention and Control

ESBL: Extended Spectrum Beta-Lactamase

ESBL-EC: Extended Spectrum Beta-Lactamase producing *Escherichia coli*

ESBL-KP: Extended Spectrum Beta-Lactamase producing *Klebsiella pneumoniae*

ESBL-PE: Extended Spectrum Beta-Lactamase producing Enterobacterales

GNB: Gram-Negative Bacteria

ICU: Intensive Care Unit

LAMP: Loop Mediated Isothermal Amplification

LTCF: Long-Term Care Facility

MDR: Multi-Drug Resistance

MDRO: Multi-Drug Resistant Organism

MRSA: Methicillin-Resistant *Staphylococcus aureus*

nEcESBLPE: non-*E.coli* ESBL producing Enterobacterales

VRE: Vancomycin Resistant *Enterococcus*

MGE: Mobile Genetic Elements

WHO: World Health Organization

CHAPTER ONE
General introduction

Part 1) Epidemiological characteristics of ESBL-PE and CPE

Global burden of Antimicrobial Resistance

The global burden of AMR, represented by 16 antibiotic-resistant bacteria combinations, currently estimates 671'689 infections, of which 63.5% are nosocomial, accounting for 33'110 deaths among European regions in 2015 (1). However, modelling approaches suffer from many limitations, including controversial attribution of death to AMR, counterfactual estimation of burden (infection by a susceptible organism vs no-infection), age-adjustment for risks, heterogeneous sampling frequencies and national coverage, and external residual confounding (2). Most of these issues, except the above-mentioned sampling, selection, and detection bias, as well as adjustment for age and gender have been addressed in a recent modelling study based on 471 million individual observation worldwide from literature and surveillance data, and estimating the excess risk of death associated with (versus deaths with no infection) and attributable to AMR (versus deaths with drug-susceptible infection) for 88 antibiotic-resistant bacteria combinations in 2019. Overall deaths associated with AMR and deaths attributable to AMR were respectively estimated at 4.95 million (95% CI 3.62-6.57) and 1.27 million (95%CI 0.91-1.71), mostly driven by lower respiratory infections. (3).

WHO list of critical priority pathogens

Aiming to guide the development of new active agents, WHO established a global priority list of 12 bacterial species with acquired resistance, selecting carbapenem-resistant *Acinetobacter baumanii*, carbapenem-resistant *Pseudomonas aeruginosa*, carbapenem-resistant and 3GCR Enterobacterales. (4) Of all 3GCR Enterobacterales, the heaviest community and hospital burden was attributed to *E. coli* and *K.*

pneumoniae. (4) This list also served to define targets for surveillance systems and outbreak reporting (5), and was used as a basis to select pathogens of high concern in this thesis. Importantly, among all resistance mechanisms, this thesis will focus on carbapenemases and ESBL. Penicillinases, AmpC beta-lactamases, and non-enzymatic mechanisms (loss of outer membrane porine) will not be considered.

General characteristics of Enterobacterales

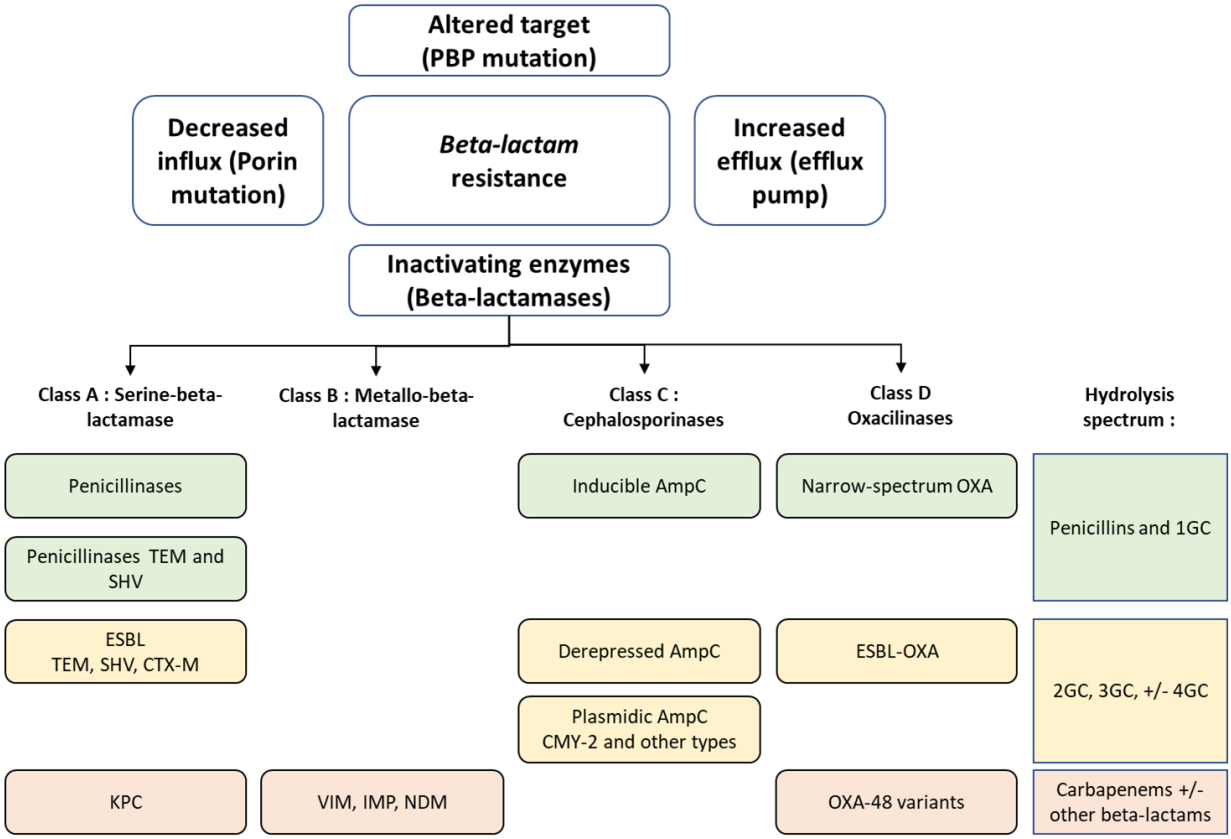
Enterobacterales are enteric pathogens colonizing the digestive tract, mostly causing a large variety of community- and healthcare-aquired infections, including cystitis, pyelonephritis, peritonitis, septicemia, and device-associated infections. The feco-oral route encompasses direct human-to-human transmission, but also indirect transmission through the environmental or animal reservoir (6,7). Human-to-human transmission has been further detailed using five sequential steps, (8) starting from (A) the presence or shedding of organisms, (B) hand contamination, (C) survival of organisms for at least several minutes, (D) inefficient hand hygiene, and (E) contamination of other patients or their surroundings. As already reviewed, these conditions perfectly apply to Gram-negative bacteria. (9)

General characteristics of ESBL and carbapenemases

As described above, ESBL-PE and CPE colonize the digestive tract, and are associated with difficult-to-treat infections. Colonization by ESBL-producing *E.coli* and *K.pneumoniae* significantly increases the risk of an infection by the same pathogen. (10,11) Similarly, the relative abundance of KPC-producing *K.pneumoniae* was also associated with an increased risk of bacteremia by the same pathogen. (12) ESBL and carbapenemases define multiple bacterial enzymes hydrolyzing respectively 3GC and carbapenems (Figure 1). These enzymes are traditionally sorted based on their amino-acid sequences with the Ambler classification. Class A enzymes (ESBL, KPC) and D (OXA-48) contain

serine in their active sites, while class B enzymes (Metallo-beta-lactamases: VIM, IMP, NDM) require bivalent metal ions, such as Zinc. Class D enzymes are known for sparing 3GC and weakly hydrolyzing carbapenems.

Figure 1. Beta-lactam resistance and inactivating enzymes



Inspired from Ruppé et al. Ann. Intensive Care. 2015;5:21

Community and hospital epidemiology of ESBL-PE

The global spread of resistance genes occurs vertically by clonal replication, but also horizontally within and between species, using

horizontal gene transfers as a nested genetic mobility within transposons within plasmids within species, with a success rate depending upon all intermediaries. (13) Such horizontal transmission is known to produce plasmid-born outbreaks within hospitals, which are challenging to detect and control. (13,14)

ESBLs emerged from mutant penicillinases (TEM and SHV) in the 1980s, mostly concerning hospital-acquired *K. pneumoniae* and *Enterobacter sp.* (15) This nosocomial predominance was explained by multiple contributing exposures, including antibiotic pressure, patients' frailty, and opportunities at risk. (16,17) An epidemiological shift of third-generation cephalosporin resistance occurred from local hospital outbreaks to widespread endemicity in the community following clonal transmission of a single ESBL-producing *E. coli* clone (O25b-ST131), (18) and horizontal gene transfer of CTX-M enzymes located on plasmids within transposons or insertion sequences. (19) This *E.coli* clone is currently spreading in Southeast Asia, Europe, and Latin-America regions. (20) Factors contributing to the widespread endemicity of this clone were importation events from international travels from endemic countries, foreign hospital transfer (21,22), but also spread from the food chain. (23) The community predominance and persistence of this clone remain unexplained, but probably result from antimicrobial resistance, clonal characteristics such as virulence factors, (24–26) and plasmid characteristics, considering the richness of IncF family plasmids in toxin–antitoxin modules. (27) These characteristics might promote a competitive advantage of both strains and plasmids against normal microbiota, despite the fitness cost of antimicrobial resistance. Though unproven, this selective advantage might promote bacterial growth and increased shedding, resulting in the observed increased cross-transmission and prolonged carriage duration observed among certain clones. (28,29) Aforementioned epidemiological shifts of 3GC-R are reflected in risk factors identified for ESBL-PE acquisition or infection

among healthy participants living in the community, including antibiotic usage, diarrhea, travels, and food-related exposures. (30)

Community and nosocomial epidemiology of CPE

Similarly to ESBL, carbapenem resistance initially emerged with localized nosocomial outbreaks of *K. pneumoniae* and *Enterobacter sp*. producing VIM and KPC. (15) Major risk factors for nosocomial CPE acquisition also reflect this nosocomial predominance, including prior antibiotic use, use of medical devices, and mechanical ventilation. (31–33) No epidemiological shift towards the community has so far been observed for carbapenemases, although they have also disseminated globally, mostly by importation events from international travel activities in endemic countries and inter-hospital transfers. (22) Spread also occurs vertically and horizontally, depending on species and plasmid characteristics. For example, the combination of horizontal transmission of KPC-2 and subsequent clonal spread of *K. pneumoniae* ST258 disseminated carbapenem resistance among hospitals. (34) Alternatively, NDM first circulated horizontally between and within species, (35) and has been more recently associated with *E.coli*. (35) Community dissemination of OXA-48 *E.coli* also has been reported in North Africa, probably by foodborne acquisition or household transmission. (36) The perfect combination of a virulent community clone with a stable plasmid, such as CTX-M-15 producing *E.coli* ST131 has not been observed yet. However, the spread of CPE warrants specific monitoring, especially when carbapenemases are combined with community pathogens such as *E.coli*.

Transmission dynamics of ESBL-PE and CPE

In-depth comprehension of transmission dynamics is required to adequately quantify and predict the spread of resistance. The analysis of transmission dynamics aims to reconstruct bacterial spread in defined populations using multiple parameters, such as acquisition rates, transmission rates, environmental persistence, and carriage duration.

Transmission dynamics differ by host-, care-, species-, and settings-related factors. Concerning hosts, antibiotic consumption, diarrhea, and open wound influence bacterial shedding, increasing microbial burden, which can easily be transmitted during patient care, by the maintenance of endotracheal tubes, wound dressings, and bathing. (37)

Concerning species, non-*E.coli* ESBL-PE have been associated with higher acquisition rates compared to ESBL-EC, with respectively 7.4 and 2.6 acquisitions per 100 admissions at risk among ICU patients. (38) Based on a mathematical model, single-admission reproduction numbers were estimated for non-EC ESBL-PE and ESBL-EC at 0.17 [95%CrI 0.094-0.29] and 0.047 [95%CrI 0.018-0.098]. (38) Despite an unclear biological explanation, a potential hypothesis is the better environmental persistence for *K.pneumoniae* as compared to *E.coli.* (39) The different profile of patients colonized by *E.coli* and non-*E.coli Enterobacteriaceae* might also contribute to this difference, with non-*E.coli* carriers more exposed to healthcare settings (e.g. febrile neutropenia, ICU). (40)

ESBL-EC transmission rates also differ among settings. In LTCF, per-admission reproduction numbers were higher, estimated for ESBL-EC ST131 and other ESBL-EC at 0.66 and 0.56 (28) Another cohort study followed roommates of ESBL-EC positive patients after discontinuation of contact precautions and observed transmission rates of 2.6% in an acute care hospital versus 8.8% in an affiliated LTCF. (41) This difference also contributes to a higher ESBL-PE prevalence in LTCF. When comparing patients hospitalized in an Italian acute-care geriatric hospital to their pairs in five affiliated LTCF units, using a cross sectional survey in 2008, prevalence of ESBL producers was 14.5% versus 64%. (42) Effectively, LTCFs have specific characteristics compared to acute-care hospitals influencing transmission dynamics. Adherence to hand hygiene (27.3% and 46.1% before and after patient care) and gloving (44.9%) is historically low in these settings. (43,44) Patients are also vulnerable to

be colonized and develop a subsequent infection, due to comorbidities, with impaired autonomy in daily care, immunosenescence, and medical devices. (45) To note, the higher transmission rates in LTCF might predominantly result from these patient characteristics compared to healthy participants in the community or younger patients in acute-care hospitals. Effectively, similar prevalence proportions were observed between LTCF residents and their pairs living in community (11% vs 8.7%). (46) However, this study possibly suffered from a selection bias, mostly including nursing homes sufficiently staffed with good hygiene practices, in low-endemicity settings (Sweden), and might not be generalizable to nursing homes in other countries.

Other settings, including households, have demonstrated significant transmission rates. Cohort studies among household settings observed acquisition and transmission rates of CPE among 9% (16/177) and 2% (3/177) of household members, with 64% and 25% of index cases being colonized by *E. coli* and *K. pneumoniae*, with difference according to the status of household members (spouse, with an OR: 6.17 [95%CI 1.05-36.35]). (47) However, the heterogeneity of study designs, outcomes, and denominators often imped direct comparison of the dynamics among these different settings.

Summary

Pathogens and associated resistances have different transmission dynamics and burden among various patients and care settings. Furthermore, heterogeneous colonization pressure and infection control policies also influence the available microbial burden and opportunities at risk for cross-transmission. Most of the available evidence defining transmission dynamics originates from outbreak investigations in specific settings, with impaired generalizability. Furthermore, studies evaluating transmission dynamics also faced several challenges, including heterogeneous definitions for transmission (phenotypic vs genotypic),

sampling bias, and detection bias. There is no single model explaining the spread of all ESBL-PE and CPE, nor a robust evidence to estimate this spread in non-ICU settings. This highlights the relevance of monitoring and further research to comprehend AMR transmission dynamics in understudied settings, such as LTCFs and household settings.

Part 2) Infection control strategies for nosocomial MDR-GNB control

Multi-faceted interventions preventing nosocomial MDR-GNB transmission

Infection control measures preventing the spread of nosocomial MDR-GNB transmission combine universal and targeted precautions, which both define two different multi-faceted (or bundled) interventions. Universal precautions include hand hygiene, personal protective equipment (PPE) whenever exposed to infectious material and body surfaces, and environmental hygiene. Universal precautions aim to prevent the nosocomial spread from resistant and susceptible organisms, from recognized and non recognized sources. Targeted precautions only concern patients with proven or suspected colonization and infection by certain infectious agents. They include isolation or cohorting, contact, droplet, and airborne precautions. Contact precautions traditionally include wearing of PPE (gloves & hydrophobic coat). Universal and targeted precautions are complemented by additional infection control strategies, such as diagnostic and antimicrobial stewardship, and potentially chlorhexidine bathing, decolonization strategies and development of novel vaccines. However, not all measures were proved to be efficient and are supported by the same level of evidence. This thesis focuses on the most effective measures to control nosocomial MDR-GNB spread in low endemic settings, excluding AMS, which are universal precautions, contact precautions, isolation, and cohorting.

Multiple guidelines offer guidance to control nosocomial MDR-GNB, including the Centers for Disease Control and the Agency for Healthcare Research and Quality (CDC and AHRQ, 2007 (48)). To note, the discontinuation of contact precautions and universal glove wearing were considered as unresolved issues. Eight years later, ESCMID offered an evidence-based guidance listing the most effective interventions to control nosocomial ESBL-PE and CPE. (9) While CDC guidelines distinguished specific settings such as LTCFs, ESCMID guidelines distinguished epidemic from endemic settings, accounting for different risks and resources. If initial recommendations concerned both ESBL-PE and CPE, guidelines specifically addressing CPE control were published later by CDC with a toolkit (2015) and by ECDC and WHO (2017). (49–51) These recommendations highlighted the importance of hand hygiene, active surveillance cultures to monitor colonization and infection, contact precautions, and patient isolation. More specifically for CPE, they also recommended patient and staff cohorting, enhanced environmental cleaning, preemptive contact precautions combined with thorough admission and contact screening, but also highlighted the importance of inter-facility communications. WHO adapted later its multimodal hand hygiene improvement strategy in 2019 to facilitate the implementation of multifaceted interventions for CPE control. (52)

Multi-faceted interventions combined universal and targeted precautions and proved to be efficient to control nosocomial ESBL-E spread. (53) Of note, most of the evidence is based from before-and-after studies occurring in the midst of outbreaks. Considering CPE, a recent systematic review and reanalysis using interrupted time series analysis observed that multifaceted recommendations using complementary infection control measures, classically active case finding, contact precautions, cohorting or isolation, hand hygiene, staffing education, and hospital hygiene, were efficient to control epidemic or endemic CRE, carbapenem-resistant *A. baumanii*, and carbapenem-resistant *P. aeruginosa*. (54)

Specifically addressing multidrug resistant *P. aeruginosa* and *A. baumanii*, represents a complex challenge because of the variability of their genome and diversity of resistance mechanisms. However, outbreak investigations in an Israeli hospital observed that such infection control measures, including cohorting, dedicated equipment and staffing can effectively control the nosocomial spread of carbapenem-resistant *A. baumanii*. (55)

Effectiveness of hand hygiene

Currently, scientific consensus considers hand hygiene as one of the core measures to control susceptible and resistant Gram-negative bacteria. However, despite robust evidence confirming hand hygiene effectiveness to control nosocomial MRSA, (56) only few studies evaluated in-vitro and in-vivo efficacy of ABHR on Gram-negative bacteria. (57,58) Kaier et al. used time-series analysis to evaluate the ecological impact of alcohol-based hand rub (ABHR) volumes on ESBL-PE incidence from 2005 to 2007, adjusting for community importation and antimicrobial consumption while keeping constant other infection control measures. (57) Though most of nosocomial ESBL-PE were influenced by community importation, ABHR volume had a negative temporal relationship with ESBL-PE incidence (6.73% decrease of ESBL-PE incidence every ABHR litres per 1'000 patient-days after 4 months). Despite hardly interpretable lags, their model explained 75% of the monthly variations of nosocomial ESBL-PE incidence. To note, no studies evaluated the specific efficacy of hand hygiene on CPE, this intervention always being included in larger multifaceted bundle approaches.

Specific challenges of contact precautions

Contact precautions imply wearing a gown and gloves upon entry in patients' rooms. It is also recommended to use dedicated or single-use non-critical care equipment when caring for patients. Preemptive contact precautions apply when these measures are implemented in the absence

of microbiological confirmation. Usually preemptive contact precautions are implemented at admission of patients at risk to be colonized and discontinued after sequential consecutive screening tests.

However, contact precautions and isolation are not trivial measures, associated with noninfectious and infectious adverse events. As noninfectious adverse events, contact precautions were observed to potentially increase the risk of depression and anxiety, but also to decrease the contact time between patients and physicians with an uncertain impact on care. (59,60) Mental health issues were explored by small underpowered studies, with findings potentially confounded by the status of MDRO carriers, more susceptible to experience co-morbidities or extended length of stay. Morgan DJ et al. (2013) covertly observed 7'743 healthcare workers over 1'989 hours, and reported 36.4% decreased hourly HCW visits rates (4.37 to 2.78 visits per hour), 17.7% decreased patient contact time (16.98 to 13.98 minutes per hour), and 23.6% fewer visitors. (61) Harris et al. (2013) also covertly observed 6'988 HCW visits in ICU during 1'473 hours, and observed a significant decrease of hourly HCW visits from 5.24 (4.46-6.16) to 4.28 (3.95-4.64) hours (p=0.02) following universal gloving and gowning for all patient contact. (62) Interestingly, both studies observed an increased adherence to hand hygiene when exiting patients' rooms (47.4% to 63.2% with contact precautions, and 62.9% to 78.3% with contact precautions). Effect of contact precautions on patient care is more controversial, with increased preventable adverse events (falls, electrolyte disorders) and worsening process of care measures (documentation of vital signs, days without a physician or nursing note). (63,64) However, larger studies using standardized tools to measure adverse events related to the quality of care (IHI Global Trigger tool) observed either no difference or fewer adverse events when applying contact precautions. (62,65) Whether this tool is sensitive enough to capture relevant adverse events and whether potentially undetected

adverse events are clinically relevant remains to be determined. Additional challenges include the limited hospital capacity in single-bed room patients, and overall costs incurred by additional isolation material, cleaning and disinfection material, additional working time, and single-use material. In 2017, these costs were estimated at 158.90$ [95%CI 124.90-192.80] following 24 hours of detailed observation of 10 patients under contact precaution in acute care wards of a Swiss University Hospital. (66)

First, gloves and gowns potentially constituting a transmission vector, infectious adverse events might occur during failures to comply with adequate doffing and donning, for example with deviations from recommendations (intentional), process or procedural mistakes (non intentional), slips or lapses (non intentional). (67) Outbreaks reporting a direct association with gloving are scarce, (68) but microbiological confirmation of glove-related outbreaks remain methodologically difficult and certainly imped reporting. Second, an inverse relationship between the number of indications and adherence to contact precautions has been observed, which might yield negative ecological consequences. In 2009, Dahr et al. conducted 1’013 covert observations HCW, and observed a dropping adherence with contact precautions from 31.5% to 6.5% when isolation burden increased from less than 20% to more than 60%. (69)

Due to noninfectious and infectious side effects, as well as considering the resources, infrastructural constraints, and costs incurred by contact precautions, adequate evaluation of their specific efficacy for each indication is important. However, such evaluation is scarce, limited by impaired generalizability, residual confounding, and detection bias. First, the generalizability of existing evidence is impaired by the number of confounding exposures related to settings, patients, and infection control measures. Effectively, contact precautions are often evaluated as a part of a bundle, which makes it difficult to disentangle the relative efficacy of

this measure. Second, traditional weekly screening schedules are insufficient to capture all acquisition or transmission events, resulting in detection bias. Admission and discharge screening should ideally be implemented in such studies. Despite these limitations, there is a growing evidence following discontinuation of universal contact precautions, targeted contact precautions for ESBL-PE, or targeted contact precautions for non-*E.coli* ESBL-PE.

Effectiveness of universal contact precautions

Effectiveness of universal gloving and gowning trials against targeted contact precautions for MRSA and VRE control has been evaluated by the BUGG study (Table 1), a cluster-controlled trial including 20 American ICUs in 2012. (62) Ten ICUs were randomized to universal gloving and gowning, and the other half was randomized to standard of care (targeted measures). Using robust methods, the authors implemented admission and discharge screening, and closely monitored acquisition rates, healthcare related infections, hand hygiene adherence, and adherence to contact precautions. To note, chlorhexidine bathing was performed in five and seven ICUs in the control and intervention arm, respectively. A later nested study of this trial specifically evaluated the effectiveness of universal contact precautions on MDR-GNB control, including 20'246 patients. (70) Following a generalized linear mixed model, universal contact precautions resulted in an overall rate ratio (RR) for MDR-GNB acquisition of 0.90 [95%CI, 0.71-1.12, p=0.34], with no specific benefits on CPE (RR 0.86 [95%CI 0.60-1.24, p=0.43], ESBL-PE (RR 0.94 [95%CI 0.71-1.24], p=0.67), carbapenem-resistant *Acinetobacter* [RR 0.81 [95%CI 0.52-1.27, p=.36], carbapenem-resistant *Pseudomonas* [RR 0.88 [95%CI 0.55-1.42, p=0.62], and with no change after adjustment for colonization pressure. This finding is supported by prior evidence, with multiple quasi-experimental studies and a mathematical model observing no change in MDRO incidence density, acquisition, and ICU-acquired MDRO infection rates. (71–74) To note, all these studies were performed

in ICU settings with low-to-medium endemicity levels (France, USA), with heterogeneous definitions for contact precautions, various screening strategies, and unclear percentage of available single-bed rooms, probably adding residual confounding. Such estimates might differ in high-endemicity settings; however in this case, hand hygiene and cohorting would probably be preferred as universal measures, considering the effect of isolation burden on adherence. (69)

Table 1. Studies evaluating universal versus targeted contact precautions to control nosocomial MDRO spread

	Design & control	Settings & No. patient	Intervention Bundle ?	MDRO	Contact precaution definition	Screening cultures	Gloves &- gowns adherence	Hand hygiene adherence	Major findings
Furuya EY et al. (2018)	QE ; historical & 3 concomittant ICU controls	3 ICU (..% single room)	I: 8Y Universal CP C: 1Y Targeted CP	MRSA VRE CRKP	…	Admission (MRSA, VRE)[a]	…	…	No significant change in hospital-acquired MDRO incidence density rate
Djibré M et al. (2017)	QE post-test control	1 ICU (100% single room)	I: 6M universal preemptive CP C: 6M targeted preemptive CP	MDRO	Glove + gown + isolation	Admission Weekly	…	…	No significant change in MDRO acquisition rate
Kardas-Sloma et al. (2017)	Dynamic stochastic transmission model	Simulated ICU	I1: 1Y CP C: 1Y HH (80% HH adherence among all ICU patients)	ESBL-PE	CP defined as 80% HH adherence among carriers	N/A	N/A	N/A	Higher overall costs compared to hand hygiene strategy
Ledoux et al. (2016)	QE ; post test control	1 ICU (100% single room)	I: 12M universal preemptive CP C: 12M targeted preemptive CP	MDRO	Glove + gown + isolation	Admission Weekly	…	Similar ABHR consumption (not shown)	No significant change in ICU-acquired MDRO infection
Harris et al. (2013)	Nested analysis of prior cluster randomized trial	30 ICUs (...% single room)	I: 9M universal CP C: 9M targeted CP	CR-PSA, CR-ABAU, ESBL-PE, CPE	Glove + gown	Admission Discharge	Gloves (I) 86.2% (C) 84.1% Gowns (I) 85.1% (C) 81.2%	(C) 62.9 – (I) 78.3%[b]	No significant change in MDR-GNB acquisition rate

[a]: Not all ICUs performed screening

[b]: Hand hygiene adherence evaluated when exiting rooms

Effectiveness of contact precautions targeting non-*E.coli* ESBL-PE

The growing prevalence and related control efforts of ESBL-EC in particular due to the community clone ST131 increasingly strained staffing resources and hospital capacity in single-bed rooms. (29) To address this issue specific to ESBL-EC, several centers attempted to discontinue contact precautions for this species (Table 2). Tschudin et al. evaluated transmissibility of ESBL-EC in a cohort study among an acute-care hospital and affiliated LTCF, using discharge screening for all contacts of an index case. (41) This study observed low ESBL-EC transmission rates in acute-care hospitals (2.6%) and LTCFs (8.8%). Similar transmission rates for ESBL-PE were observed in the same acute-care hospitals during implementation of contact precautions for all ESBL-PE (1.5% among 133 contact patients). (75) Authors also observed similar rates among other acute-care hospitals and LTCFs. (41) Interestingly, ESBL-PE positive roommates had superior contact time compared to negative roommates (median 13 days (IQR 10-15) vs 8 days (IQR 5-12), p=0.006). (41) In 2015, Biehl et al. observed similar results in a prospective cohort study including 1'386 and 1'582 patients from two hematology and oncology sites, respectively, implementing single-room contact precautions in addition to standard precautions for F3GCR-EC. (76) Admission and discharge screening were complemented by whole genome sequencing to ascertain transmission events. Despite the large sample size and the robust screening strategy, only three transmission events were observed. Interestingly, the authors estimated the number of patients needed to screen to prevent one transmission event at 3'729. Another cohort study performed by Zahar et al. retrospectively compared ESBL-EC incidence between two French hospitals from 2006 to 2010, one implementing standard precautions and another implementing contact precautions targeting ESBL-EC. (77) Concomitant increase in ESBL-EC incidence was observed in both intervention and control groups, without any clear difference. However, the different age of patients (median age

of 61 vs 9 years-old patients) hospitalized in interventional and control hospitals might have confounded the effect of contact precautions. Nevertheless, sufficient evidence with reproducible results is now available from different healthcare settings to support the discontinuation of contact precautions for ESBL-EC.

Table 2. Studies evaluating contact precautions targeting non-*E.coli* ESBL-PE vs ESBL-PE

	Design & control	Settings & No. patient	Intervention Bundle ?	MDRO	Contact precaution	Screening	Gloves &-gowns adherence	Hand hygiene adherence	Major findings
Biehl et al. (2019)	Prospective cohort study	4 (2xI & 2xC) haematological and oncological departments	I: 12M CP C: 12M SP	F3GCR-EC	Single room + glove + gown	Admission + discharge	..	>80%	Same rate of hospital-acquisition and transmission
Tschudin et al. (2016)	Prospective cohort study	Acute care hospital (91.3% rooms with 1-2 beds) LTCF (52.5% rooms with 1-2 beds)	18M: no CP for ESBL-EC	ESBL-EC	…	Contact screening (once before discharge, swab & cultures)	…	>90%	Transmission with 2.6% (acute care) and 8.8% (LTCF) of contacts.
Zahar et al. (2015)	Retrospective cohort study	2 (I & C) University Hospitals	5Y: CP for ESBL-EC 5Y: SP for ESBL-EC	ESBL-EC	Private room + dedicated material + gown for long and close care	Admission and weekly screening[a]	…	Similar consumption of ABHR	No significant change in ESBL-E incidence between the two hospitals

[a]: only in ICUs. Only clinical cultures were considered in the ESBL-E incidence

Effectiveness of contact precautions targeting ESBL-PE

As detailed above, certain species such as *K.pneumoniae* may spread more easily. However, some centers discontinued contact precautions not only for ESBL-EC but for all ESBL-PE regardless of the species, and provided the opportunity to evaluate the specific effectiveness of contact precautions for all ESBL-PE (Table 3). (75,78–80) The largest available trial is a cluster randomized cross-over trial (R-GNOSIS) including 11'368 patients screened at least twice from 20 non-intensive care units in four hospitals from 2014 to 2016. (78) This trial aimed to compare contact and standard precautions against standard precautions to control ESBL-

PE acquisition events, using admission, weekly, and discharge screening. Maechler et al. also collected several meaningful confounders, such as antibiotic consumption, colonization pressure (ESBL-PE burden at admission), screening rates, and hand hygiene adherence, which were all similar in both periods. Incidence densities of ward-acquired ESBL-PE were not different in interventional vs control periods (6.0 [95%CI 5.4-6.7] versus 6.1 [95%CI 5.5-6.7] per 1'000 patient-days at risk). This finding did not change after adjustment for length of stay, screening rate, and colonization pressure. Interestingly, the isolation burden significantly decreased during the control phase from 58.9% to 11.2% of patients under contact precautions, but among them, adherence with isolation in a single-bed room drastically increased from 50.3% to 81.4%. We can hypothesize that increased availability of single-bed rooms contributed to a reallocation of resources toward more critical patients. Other studies adopted a quasi-experimental design, were implemented in ICUs and non-critical wards, had various screening strategies and definitions for contact precautions, and did not systematically report hand hygiene adherence. (79,80) Tschudin et al. described in a cohort study very low transmission rates of ESBL-PE among roommates of a positive carrier (n=579) despite a median unprotected time of 3 days (range, 1-37 days). (75) Despite this heterogeneity, the same conclusion was reproduced by several studies with different study designs and patient populations, which supports the discontinuation of ESBL-PE in critical and non-critical wards in low endemicity settings, under the condition of high adherence to standard precautions.

Yet, multiple factors should be considered before deciding to discontinue contact precautions for ESBL-PE. According to Lucet et al., (81) patient-, epidemiology, infection control-, and resource-specific data have to be considered to individualize this decision making. Potential patient-level exposures include shedding high bacterial loads (diarrhea, UTI, wounds), for patients requiring intensive care (increased opportunities at risk), for vulnerable patients (e.g. transplant units). Epidemiological and

microbiological exposures would include epidemic settings, extensively resistant pathogens, ease and route of transmission. Infection control information includes hand hygiene adherence and active surveillance cultures. Resource-related information includes hospital infrastructure (crowded multiple-bed rooms, availability of single-bed rooms), human workforce (dedicated staffing, additional time for donning & doffing), and financial resources.

Table 3. Studies evaluating contact precautions for ESBL-PE

	Design & control	Settings & No. patient	Intervention Bundle ?	MDRO	Contact precaution	Screening	Gloves &- gowns adherence	Hand hygiene adherence	Major findings
Maechler F et al. (2020)	Cluster randomized crossover trial	20 non intensive care units	C: 12M SP I: 12M CP	ESBL-E	Isolation (when possible) + glove + gown	Admission + weekly + discharge	Gowns I: 84% Gloves I: 89%	C: 61% I: 62%	Similar ESBL-PE incidence density
Thompson et al. (2019)	QE ; post test control	1 University hospital	I: 22M CP C: 20M SP	ESBL-E	…	No screening	…	…	Increased incidence of healthcare associated ESBL-PE infections with CP
Renaudin et al. (2017)	QE ; post test control	1 ICU (100% single room)	I: 25M CP C: 25M SP	MRSA ESBL-PE	Isolation + gown	Admission + weekly	75%[a]	81%[a]	Lower incidence rate of ICU-acquisition in SP period
Tschudin et al. (2012)	Prospective cohort study	University hospital	I: 12Y SP[b]	ESBL-PE	N/A	Contact screening	N/A	…	Transmission with 1.5% of contacts.

Effectiveness of contact precautions targeting CPE

The current effectiveness of contact precautions targeting CPE has been scarcely described, due to its continuous and almost mandatory application among healthcare facilities. A retrospective cohort study including multiple hospitals totalizing 21,000 beds from 2010 to 2015, described multiple importation events associated with a subsequent outbreak (involving at least one secondary case among contact patients with a defined epidemiological link and with similar species and resistance genes). (82) Upon 655 importation events at admission, 51 (8%) were followed by an outbreak. If implemented in the two days following admission of the index case, contact precautions had a protective effect

(OR 0.41 [95%CI 0.22-0.74], p<0.001). Thus, contact precautions as part of the interventional bundle to control nosocomial CPE seems effective. To note, *K. pneumoniae* was again associated with a higher risk to generate outbreaks (OR 4.98 [95%CI 1.16-21.45]). Furthermore, CPE importations remain associated with large-scale hospital outbreaks, with significant human and economic cost, estimated at €1.1m (range 0.9-1.4) for a 10-months outbreak. (83) In summary, considering the significant burden of CPE and their association with large scale nosocomial outbreaks, no attempt in discontinuing contact precautions has yet been recommended in any national or international guideline. Even infection control nihilists still continue to advocate active screening and contract precautions for CPE control, despite weak evidence and absence of controlled trial data.

Effectiveness of patient or staff cohorting, and isolation in single-bed rooms

Current recommendations suggest to isolate non-*E.coli* ESBL-PE and CPE carriers in endemic settings, and to cohort patients and staff in epidemic settings. (9) The additive effect of isolation in single-bed rooms on effectiveness of contact precautions has recently been quantified. In a large cluster-randomized, cross-over study including 16 Dutch hospitals from 2011 to 2014, Kluytmans et al. compared the efficiency of contact precautions in single-bed room versus multiple bed-rooms to control ESBL-PE. (84) This study included a total of 312 and 304 index patients for respectively 4'790 and 4'578 roommates for both single-bed and multiple-bed room strategies. To note, 88% and 62% of index patients were adherent to the assigned strategy. When regarding per-protocol populations, ESBL-PE transmission rates to at least one roommate were similar between single-bed and multiple-bed room strategies (crude risk difference 3.4% [90%CI -0.3-7.1]). Though data on complementary infection control measures were not collected (hand hygiene adherence), and despite a low adherence to multiple-bed room strategy, this study

used a robust design and statistical methods to observe no difference between contact precautions with isolation versus contact precautions without isolation. However, this study was not able to include a third group without contact precautions, but observed an increased risk of ESBL-PE transmission with unprotected ward stay. These findings were supported by another study by Repessé et al. evaluating the effectiveness of contact precautions without isolation on ESBL-PE transmission rates, using an ICU with only twin-bed rooms. (17) To note, gloves were not included in the definition of contact precautions. Despite the absence of single-bed rooms, the authors observed 4.1% ESBL acquisition and only 2 cross-transmission events among 470 patients from 2014 to 2015. In contrast, Prevel et al. observed in 2015 only 1% ESBL-E acquisition event and only 1 ESBL-E cross-transmission event among 608 ICU patients screened at admission and weekly in ICU applying contact precautions and isolation for all known ESBL-E carriers. (85) The reproducibility of these studies, along with the low frequency of ESBL-PE transmission events in hospitals argue against an additive effect of isolation during application of contact precautions in non-epidemic settings.

Table 3. Studies evaluating the additive effect of isolation on contact precautions

	Design & control	Settings & No. patient	Intervention Bundle ?	MDRO	Contact precaution	Screening	Gloves &- gowns adherence	Hand hygiene adherence	Major findings
Kluytmans-van den Bergh et al. (2019)	Cluster-randomized cross-over study	16 hospitals	I1: CP in multiple bed-room I2: CP in single bed-room	ESBL-PE	Glove + gowns	Baseline weekly	…	…	Similar ESBL-PE transmission rates
Repessé et al. (2017)	Prospective cohort study	1 ICU (0% single room)	C: 11M CP	ESBL-PE	Gown	Admission Weekly	…	135-137 L/1'000 patient days	4.1% ESBL acquisition & 2 transmission events

Considering CPE control, the importance of patient isolation or cohorting has been strongly emphasized, despite the scarcity of evidence. A selection of outbreak investigations reporting staggered interventions

failed to control the spread until cohorting of patients and staff. (86) In Israel, the implementation and strict adherence to isolation of CR-*K. pneumoniae* carriers successfully controlled large institutional outbreaks, (87) and decreased monthly nationwide incidence of clinically diagnosed CRE carriers from 55.5 patients per 100'000 patient-days to 11.7 patients per 100'000 patients-days (p=0.001). (88) This measure should apply to patients and staff, and ideally would include dedicated medical devices. Cohorting CRE patients is more appropriate in epidemic settings, and proved to be efficient in reducing the rate of CRE acquisition in highly endemic settings. (89)

Effectiveness of alternative MDRO control measures

While complementary infection control approaches are outside the scope of this thesis, they might also contribute to control nosocomial ESBL-E and CPE spread, including AMS. A recent retrospective study evaluating the impact of a comprehensive hospital-based AMS on healthcare-associated versus community-associated MDRO using interrupted time series analysis, observed that the mean monthly incidence of HA-MDRO infections decreased by 13% (IRR 0.87 [95%CI 0.73-1.04]), while CA-MDRO simultaneously increased by 68% (IRR 1.68 [95%CI 1.57-1.82]). (90) Though this intervention might have failed by itself to prevent spread of MDRO, (48) it probably contributed to the overall effect from bundled interventions and remains recommended in several guidelines. (9,48) However, AMS benefits might depend on the type of AMS implemented, species and resistance considered. (91)

Hospital hygiene with adequate environmental cleaning also participates in controlling nosocomial MDRO spread, though evidence is currently insufficient to quantify its importance for MDR-GNB. (92) Aquatic reservoirs and especially hospital sinks are increasingly recognized in waterborne outbreaks. From 2014 to 2017, 134 of 620 (21.6%) consultations with 1'380 patients involved transmission of water-related

organisms in healthcare. (93) Splashing effects to medication preparations were identified as a frequent pathway.

Finally, ESBL and CPE decolonization by topical antibiotic regimens has been suggested as possible additional measure, as done for MRSA decolonization for several decades. However, hypothesized benefits of decolonization regimens for 3GCR-E and CRE carriers have been refuted by several recent randomized controlled trials (94). Thus, based on an exhaustive systematic review, current guidelines do not recommend using decolonization regimens for these pathogens. (95)

Summary

In summary, although bundled preventive interventions are efficient to control CPE and ESBL-E, specific benefits from each individual component remain poorly studied and influenced by various species-, patient-, care-, organizational-, and epidemiological factors. Evidence is encouraging but remains scarce when discontinuing contact precautions in different settings (e.g. LTCFs, highly endemic settings) for all ESBL-PE. Considering the burden related to CPE, and large scale CPE outbreaks reported in hospitals, disentangling efficient bundled interventions by discontinuing contact precautions is not an option for CPE.

Part 3) The role of active surveillance in preventing nosocomial MDR-GNB cross-transmission

Active surveillance remains a core component of bundled interventions for controlling both epidemic and endemic nosocomial MDR-GNB. This surveillance can be either targeted or universal, implemented at admission or by regular weekly screenings. Few hospital units even

perform discharge screening. Active surveillance yields individual and ecological benefits. At the individual level, the surveillance supports timely and adequate implementation of infection control measures. It may also allow more appropriate empiric therapy in MDRO carriers with clinical infections, or may help to adjust perioperative surgical prophylaxis. At the ecological level, surveillance cultures may help to monitor the local MDRO epidemiology, unveil silent outbreaks, and contribute to improved care by monitoring the effect of interventions. Surveillance can be implemented universally among all hospitalized patients at admission and by a regular basis (weekly), or targeted among patients presenting predefined risk profiles. This surveillance can be implemented either institutionally or in high-risk units such as ICUs.

Effectiveness of universal screening to control nosocomial ESBL-PE and CPE

Targeted vertical control measures based on universal screening were compared against universal horizontal control measures to control MDR-GNB by Derde et al. using a cluster randomized trial among 13 ICUs. (96) Enhanced hand hygiene (adherence, 77%), chlorhexidine bathing, and contact precautions were compared to a strategy of universal admission and weekly phenotypic screening and contact precautions targeting known carriers. No significant difference was observed between both phases in steps and trend changes for MDRO acquisition (weekly IRR 0.63 [%95CI 0.35-1.15] and 1.02 [95%CI 0.99-1.03]). Thus, universal surveillance screening failed to improve ESBL control in ICUs. However, alternative transmission pathways such as environmental reservoirs were not considered and could have contributed to MDRO acquisitions. Another study attempted to quantify the specific benefits from universal surveillance, by Jalalzai et al., which discontinued universal admission and weekly screening in a single ICU, including 524 and 545 patients in the surveillance and non-surveillance phases during two periods of 12 months. (97) The authors observed no difference regarding ICU-acquired

ESBL-PE infections (1.1 versus 1.5%, p=0.64), but a decrease in antibiotic consumption during the non-surveillance phase (75 to 61 carbapenem days per 1'00 patient-days, p=0.01). However, if these two studies advocate against universal surveillance screening to control ESBL-PE, they may have overlooked some of its significant side-benefits.

Effectively, active surveillance can contribute in unveiling hidden reservoirs, ultimately improving epidemiological understanding of unknown transmission chains. Otter et al. implemented enhanced CPE screening, with resulting increased number of screenings (4'530 to 10'589 from July 2015 to March 2018), but similar proportion of positive screening (0.4%) and an increased rate of CPE detection. (98) These findings suggested prior under-detection of CPE acquisitions and contributed to guiding more aggressive infection control policies. Another side-benefit is the detection of institutional cross-transmission events, facilitating early outbreak management. Also, regular screening can improve the detection of certain cases previously missed at admission. An increased detection rate of Gram-negative bacteria carriage has been observed in early hospitalization days for unclear reasons, probably due to unmasked carriage following antibiotic treatment, or nosocomial acquisition. (99)

Effectiveness of targeted screening to control nosocomial ESBL-PE and CPE

Targeted screening specifically focuses on patients presenting defined risk profiles, which can be similar for both ESBL-PE and CPE carriers. Traditional risk profiles consider the previous exposure to healthcare settings, transfer and repatriation from abroad, prior antibiotic exposure, known carriage, and prior procedures (dialysis, invasive procedures) (31,50,100) The risk from prior healthcare exposure depends on the epidemiological situation of the healthcare facility and country. (22) Travels in foreign countries with high endemicity might also constitute a

risk depending on patient characteristics (immunosuppression, co-morbidities) and exposure related to this travel (hospital, diarrhea). (50) However, the complexity of risk profiles might result in a significant information bias, which requires to simplify history taking by including only the most significant exposures (i.e. overnight stay in a healthcare setting, hospitalization abroad (22)).

Targeted screening was compared against universal screening by Dananché et al. by a quasi-experimental study with concomitant controls. No increase in 3GCR-E related healthcare-associated infection incidence rates was observed after replacing universal screening by screening targeting patients transferred from other units or hospitals. (101) However, targeted screening depends on risk profiles considered and adherence of healthcare workers to thoroughly extract the relevant information. Lusignani et al. observed in a retrospective case-control study from 2011 to 2016 in a European academic hospital using admission screening among patients at-risk that 37 (63.8%) of the 58 CPE carriers were not identified by their risk-based screening (0.12/1000 admissions were CPE carriers). (102)

The importance of sequential screening

Importation events are mostly driven by a subpopulation of patients either hospitalized abroad or known for prior carriage, with a high pre-test probability to be colonized. For example, an observational study assessing MDRO prevalence among patients hospitalized abroad between 2010 and 2019 observed that colonization rates in patients transferred from Asia were 71.9% (69/96) versus 18.9% (99/524) from Europe. (22) Overall, 23% (163/698) were colonized by ESBL-E, and 2% (14/698) by CPE. Prevalence was even higher when these patients had prior ICU stays or antibiotic treatments. Thus, among patients with hospitalization abroad, up to two sequential screening cultures may be recommended to account for the risk of false negative results, originating from multiple

factors, including pre-analytical (sampling quality), and analytical determinants (test under-performing, bacterial load under the detection threshold in patients under efficient antibiotic treatment). Similarly, sequential cultures are also required to report decolonization of known ESBL-E and CPE carriers. In this context, up to five negative screening cultures may be recommended. ESCMID guidelines recommended to stop contact precautions after at least three consecutive negative screening cultures targeting the organism over a week or two among patient not receiving antibiotics. (9) However, considering the complexity of factors influencing both risk profiles and diagnostic performances, an ECDC guidance suggested to act at a case by case basis (at least for known carriers). (50) The indication and methods defining sequential screenings remain largely arbitrary and could benefit from further research. Sequential screening practices are thus highly heterogeneous among countries and might consequently delay the time under contact precautions or isolation, and increase screening-related costs. To note, the pertinence of sequential screening samples among known carriers is intrinsically related to the clearance of carriage, which depends on re-acquisition events, and the definition of carriage duration, frequency of intermittent or persistent carriage, as it was previously defined for MRSA. (103) However, there is not a single screening strategy adequate for all types of MDR-GNB and patients. Furthermore, risk factors for intermittent or persistent carriage are not well defined yet, and might depend on patient and setting characteristics.

Summary

In summary, although universal, systematic active surveillance screening is not efficient to control MDR-GNB acquisition and transmission rates compared to targeted screening, its side-benefits should not be ignored, including fortuitous detection of institutional clusters, and epidemiological monitoring of pathogens of concern. Targeted screening remains a core measure to control importation events, but is highly dependent on the

correct, rapid, and exhaustive identification of the subpopulation targeted. Sequential screening for certain subpopulations at high risk remains an important but understudied parameter.

Part 4) The role of diagnostic stewardship programs in active surveillance screening

Screening strategies might be complemented by diagnostic stewardship, which is defined according to GLASS as the "*coordinated guidance and interventions to improve appropriate use of microbiological diagnostics to guide therapeutic decisions. It should promote appropriate, timely diagnostic testing, including specimen collection, and pathogen identification and accurate, timely reporting of results to guide patient treatment*." (104) Diagnostic stewardship encompasses pre-analytical, analytical, and post-analytical processes to contribute to more rapid, robust, and actionable diagnostic procedures. This includes the identification and optimization of target populations, sampling methods and processing, the selection of appropriate screening tests, and the timely notification and interpretation of accurate results.

Identification of patients at-risk in active surveillance screening

The rapid identification of candidates for admission screening also accelerates the delay before screening and overall turn-around times. Based on several outbreak reports and following ESCMID recommendations, readmission alerts are currently suggested to facilitate identification of known carriers. (9) Other risk factors are either standardized (overnight stay in a healthcare facility) or dynamic and assessed with IPC specialists (e.g. transfer from a unit with a reported outbreak). Though not included in current guidelines, an evidence-based list of relevant risk factors, which need to be regularly updated, should contribute to rapid and adequate admission screening. Ideally, these risk

factors should be simplified and harmonized to facilitate the early identification of patients at-risk.

Sampling methods in active surveillance screening

Several pre-analytical factors influence the diagnostic performances and turn-around-times until result notification. Such factors include screening sites and sampling methods. Regarding screening samples, intra-anal or rectal swabs are usually preferred to stool cultures or peri-anal swabs to facilitate sampling and processing, and to collect more fecal material. Effectively, rectal sampling was more performant compared to perianal sampling. (105) To note, the performance of peri-anal swabbing is possibly influenced by gender and species, with higher yield observed in males and higher performances for *A. baumanii*. Furthermore, screening samples are not always indicative for colonization at other body sites and should be complemented by clinical cultures when indicated.

A prospective cohort study sampled at various sites (including skin, nasopharynx, urine, rectum, and wounds) all inpatients with ESBL-producing organism related infection. Eighty-eight among 100 patients had no positive clinical cultures outside the primary site of infection. (106) Considering sampling methods, polyurethane-cellular-foam and nylon-flocked swabs observed superior recovery compared to classical rayon swabs used. (105)

Phenotypic and genotypic methods for active surveillance screening

Antimicrobial resistance can either be assessed phenotypically by using Minimal Inhibitory Concentrations (MIC) or genotypically by identifying resistance genes. ESBL and CPE screening methods classically follow a multi-step hybrid strategy based on both phenotypic and genotypic tests, including screening culture, confirmation testing, pathogen identification, and antibiotic susceptibility testing.

Screening cultures are most frequently performed using selective agar supplemented by antibiotics. Addition of chromogens targeting specific enzymes (β-galactosidase, β-glucuronidase and deaminase) allows the early identification of certain pathogen groups, such as ChromID ESBL (Bio-Mérieux, France) or Brilliance ESBL agar (Oxoid, UK) with observed sensitivity values of 97.5% and 98.6%. (107) Selection of CPE is more challenging regarding the various hydrolytic activity of carbapenemases. For instance metallo-beta-lactamases (e.g. NDM, VIM) have a high and broad enzymatic activity, while OXA-48 poorly hydrolyzes carbapenemases and spares 3GC (e.g. ceftazidime). Additional selective agar were developed for CPE (Brilliance CRE (Oxoid, UK), chromID CARBA and chromID OXA-48 (bioMérieux, France), and McConkey agar supplemented with ertapenem and cloxacillin). ESBL selective media can also contribute to CPE screening, with 3GC hydrolysis by carbapenemases, though they have a reduced sensitivity. (107) Diagnostic performances of selective ESBL and CPE media are particularly low for OXA-48, which requires a specific medium (e.g. ChromID OXA-48). (108) The susceptibility profile of each isolate for different antibiotic classes is then measured by disc diffusion method using defined MIC breakpoints (either EUCAST or CLSI), commercially available semi-automated assays (Vitek 2, BioMérieux ; Phoenix, BD Diagnostics), or broth micro-dilution. To note, CLSI breakpoints have recently been lowered to improve sensitivity of tests. (109) Phenotypic confirmation testing for ESBL includes double-disc synergy tests (DDST) and combined disc tests (CDT), which often uses beta-lactamase inhibitors to preclude non-ESBL mediated resistance (hyperproducing K1 penicillinases and high level AmpC production). Phenotypic confirmation testing for carbapenemases also uses specific inhibitors. Modified Hodge tests have been historically used as confirmation assay, but were recently discarded due to poor diagnostic performances. (107) Biochemical confirmation testing for ESBL and CPE might use colorimetric tests, using a pH indicator on isolates to detect carboxylic acid following hydrolysis

reactions. ESBL-NDP and Carba NP tests observed adequate sensitivity and specificity on isolates. (107) Genotypic ESBL and CPE confirmation testing can also be performed by the characterization of targeted resistance genes present in samples or subsequent isolates using multiplex PCR, microarrays, or Loop-Mediated Isothermal Amplification Assay (LAMP).

Phenotypic methods are efficient and inexpensive for detecting the most frequent pathogens. (110) However, their performance remains dependent on the sampling quality, (111) and their results are often delayed by 36-48 hours. (112) Thus, current microbiologic culture methods are slow and not adapted to the rapid turnover in busy ICU settings. Molecular methods address some of these pitfalls, sparing the culturing effort, and resulting in a reduced turn-around time. (113,114) Genotypic methods are more discriminant, and thus more useful in informing epidemiology and infection control, helping to ascertain cross transmission events. They can also decrease the required volume of sampling because of their improved sensitivity. (115) However, molecular methods also suffer from several limitations. First, their breadth is limited to the selected molecular targets, which could result in insufficient coverage of emerging or rare resistance genes. Second, the poor specificity might impact the clinical pertinence of notified results. Third, their cost-effectiveness is still unclear, but might become attractive when compared to the cost of unnecessary isolation. (116)
In order to improve effective ESBL-PE control strategies in the ICU setting, there is a need for a fast, sensitive, and reasonably specific but also cost-effective screening test.(117) Currently, most of genotypic methods are validated and performed on isolates, reducing the expected time benefits. However, effectiveness of multiplex PCR, and Loop-Mediated Isothermal Amplification Assays (LAMP) were recently measured when directly applied on screening specimen.

Rapid screening strategy based on multiplex PCR applied on screening specimen

Recent studies observed poor positive predictive values when comparing multiplex PCR directly performed on screening specimen against selective media. Engel et al. evaluated the Check-Direct ESBL Screen for BD MAX (Check-Points Health BV, Netherlands) directly on 573 rectal swabs from Dutch hospitals and compared with a combination of culture (Brilliance agar) and Check-MDR CT103XL (Check-Points). (118) The qPCR assay yielded poor positive predictive values varying between 58.3% and 84.2%, with 26 discordant results (8 culture positive and qPCR negative, and 18 culture negative, qPCR positive). Another study by Jin Ko et al. compared positive Xpert Carba-R assays (Cepheid, USA) against chromID CARBA media (bioMérieux, France) for CPO detection among 30 admission screenings in ICU patients in South Korea. The authors found a positive predictive value of 53.6% [95%CI 40.4-66.4] for this test. (119) However, no study evaluated the clinical effectiveness of using a multiplex PCR directly on screening specimens to accelerate the implementation of infection control measures.

Rapid screening strategy based on LAMP applied on screening specimens

LAMP is a molecular amplification method using a DNA polymerase, Bst polymerase, providing self-replication and strand displacement through the formation of a loop with the help of 4 primers spanning 6 locations on the original DNA target. (120) Details of the LAMP method can be found at: http://loopamp.eiken.co.jp/e/index.html. (121) This technique does not use thermal cycles as PCR (122) and its enzyme is less susceptible to inhibitors than the Taq polymerase, (123,124) faster, (122) and as sensitive (125) and specific as home-made qPCR assays. (124) As a basis, the LAMP technology has already proved to be robust, (124) cost-effective, (125) speedy (124,126) and performant for detecting ESBLs and carbapenemases on screening isolates. (127) The

specific LAMP Eazyplex Superbug CRE assay also showed solid performances in the literature for the detection of various ESBLs- and CPE on different types of isolates. (128) A UK study compared in 2015 the diagnostic performance between the Eazyplex SuperBug Complete A kit performed on the GENIE II platform with a reference standard using PCR assays and a commercial microarray (Check-MDR CT102) on 450 clinical isolates with various bacterial species. The overall test sensitivity and specificity were reported as 95.5% and 100%, respectively, although it missed the detection of 18/102 OXA-48 variant carbapenemases genes. The delivery of a modified test "Eazyplex SuperBug complete B kit" resolved later this issue and identified the 18 OXA-181 producers.(129) Another Spanish study compared the Eazyplex SuperBug CRE kit performed on the GENIE II platform with phenotypic methods to identify carbapenemases and ESBLs, but also with conventional PCR assays and sequencing to characterize these enzymes. This study performed on 94 genotypically characterized carbapenemase-producing strains and 45 clinical isolates observed a 100% agreement between the Eazyplex SuperBug CRE system results and the PCR and sequencing results. Another 100% agreement was found between the inferred phenotype of clinical isolates and the Eazyplex SuperBug CRE system results.(127) LAMP demonstrated similar performances on isolates and cultures, for the direct detection of *E.coli* on urine samples.(123) To date, the only study evaluating effectiveness of LAMP to inform infection control measures was recently published by Yamamoto et al. (130) in a quasi-experimental study from Thailand to control carbapenem-resistant *A. baumanii* in ICUs. During 3-months observational and 9-months interventional periods including respectively 187 and 866 patients, and using an universal admission, weekly, and discharge screening (rectal swab & bronchial aspirates), the authors implemented contact precautions based either on culture results (control period) or LAMP results (interventional period). The implementation of LAMP tests was associated with a decreasing incidence rate of CRAB infection from 35.2 to 20.9 per 1'000 patient-days

(p<0.02). However, the positive predictive values of this test when performed on bronchial aspirates and rectal swabs remained weak, with respectively 65 % [95%CI 56-73%] and 62% [95%CI 55-69%]. Furthermore, the effect from enhanced screening implemented in the control period could have participated in decreasing the incidence rate during the interventional period. Additionally, the high-endemicity of *A. baumanii* in this setting might impair the generalizability of findings.

Summary

Performances and turn-around-times of the screening strategy are highly impacted by pre-analytical and analytical parameters, including the methods and delay to identify the target population, the selection of sampling methods, sampling sites, and screening tests. Phenotypic tests remain superior for detecting emerging resistance, by delivering key information, such as viability, linking resistance and species identification, and phenotypical susceptibility, but remain slow and delay timely infection control measures. Genotypic tests are rapid and sensitive to identify epidemiologically-relevant information, but have a poor positive predictive value when applied directly on screening specimens and their effectiveness to support infection control measures remains understudied. Nevertheless, there is potential that genotypic surveillance methods might deliver actionable results to accelerate infection control measures in certain settings, such as ICUs.

Part 5) The importance of standardization in surveillance and infection control measures

Indications and application of active surveillance cultures is highly heterogeneous within and between countries. Pathogens targeted by screening vary, with only 21.9% on 329 German hospitals reporting ESBL screening in 2014. (131) Screening strategies also depend on varying risk profiles considered for targeted screening. In The Netherlands, four

among 18 (22.2%) hospitals did not implement admission screening for MDR-GNB targeting patients with an overnight stay in a foreign hospital. (132) As described above, if certain exposures are changing over time (e.g. reported outbreak in a unit), others are constant and could be homogenized and simplified using evidence-based standardized lists. This would ultimately influence sensitivity and specificity of screening strategies but also incurred costs. Variation of screening tests was also observed by Berry et al. in 2016 after surveying acute National Health Service hospital trusts in England. All hospitals performed CPE screening using rectal swabs, but screening tests varied with selective agar, molecular, and other techniques in 76%, 4% and 20% of the cases. (133) Half of hospitals performed local confirmatory CPE testing, among them 56% used phenotypic methods, while others used biochemical or genotypic methods. Echoing these findings in 2019, Tschudin-Sutter et al. observed a consensus between Swiss, German and French tertiary care centers to use phenotypic screening cultures. (134) However, the German center was not using NAAT-based diagnostic approaches for CPE, preferring to provide phenotypic criteria of resistance.

Variation of infection control measures was also observed with heterogeneous definitions of contact precautions among studies. Similarly, patient isolation may differ by (1) placing the patient in multiple-bed rooms, (2) placing the patient in single-bed rooms without designated personnel, (3) placing the patient in single-bed rooms with designated personnel, (4) cohorting all colonized patients with designated personnel. Coppéré et al. observed among 73 French ICUs in 2016 that preemptive isolation at admission was implemented for 60 (82%) ICUs, and only 42 of them (71%) implemented targeted preemptive isolation among patients at-risk. (135) Few ICUs included gloving in the definition of contact precautions, with 18 (25%) and 38 (52%) requiring gloves at room entry or before patient contact. Gown use was defined as part of contact precautions at room entry or before patient contact in 30 (41%)

and 67 (92%) of ICUs. Heterogeneous measures were also observed by Vuichard Gysin et al. when questioning infection control specialists across 213 European (EU) and non-EU countries. Twenty-three percent and 35% of EU and non-EU countries discontinued contact precautions for non-*E.coli* ESBL, and more alarmingly 8.2% and 18.4% of EU and non-EU hospitals did not implement contact precautions for carbapenem resistant non-*E.coli.* (136) The insufficient number of isolation rooms was one of the major encountered barriers impeding correct implementation of isolation. This heterogeneity highlights the need for stronger evidence to build a consensus and homogenize practices to improve MDR-GNB control in the healthcare sector.

Part 6) Thesis objectives and specific aims

This thesis aimed to better understand the transmission dynamics and temporal trends of Gram-negative resistant bacteria, and more specifically of ESBL-producing *E. coli* and *K. pneumoniae* in understudied settings, such as long-term care facilities and the community. Secondly, this thesis aimed to improve active surveillance screening strategies by measuring existing gaps and barriers, and by evaluating innovative screening methods in accelerating infection control measures and controlling MDR-GNB among high-risk patients.

Specific aims for this study include :

ESBL-PE epidemiology in the community

- To assess the proportion of co-carriage and transmission of ESBL-producing *E.coli* and *K.pneumoniae* among household members.

ESBL-PE epidemiology in a long-term care facility

- To assess the temporal trends in the prevalence of ESBL-EC clones in a long-term care facility

- To estimate the epidemic potential of emergent ESBL-EC subclones in a long-term care facility

Implementation and efficacy of screening strategies to control antibiotic-resistant Gram-negative bacteria

- To evaluate current MDRO admission screening practices in Swiss hospitals and barriers impeding their implementation
- To compare traditional phenotypic methods with rapid screening strategies to accelerate the discontinuation of unnecessary preemptive CP for negative patients screened at admission, and the implementation of infection control measures for newly identified carriers.

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CHAPTER TWO

Nosocomial and community epidemiology of ESBL-producing *E. coli* and *K. pneumoniae*

Part 1) Epidemiology of ESBL-producing *Escherichia coli* from repeated prevalence studies over 11 years in a long-term-care facility

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Abstract

Background:

Escherichia coli sequence type (ST) 131 H30 is an emerging multidrug resistant subclone, known to spread and cause outbreaks in long-term care facilities (LTCFs).

Objectives and Methods:

From 2010 through 2020, we performed 11 yearly surveillance studies for determining the prevalence of digestive carriage of ESBL-producing *E. coli* (ESBL-EC) among residents in a university-affiliated LCTF. Sequencing and genotyping of selected isolates were performed to characterize temporal trends in the prevalence and epidemic potential of ESBL-EC subclones, and for evaluating a potential rebound effect following discontinuation of contact precautions for ESBL-EC carriers in January 2019.

Results:

This study included 2'403 LTCF residents, with 252 (10.5%) positive for ESBL-EC. Among the 236 ESBL-EC isolates available for typing, 58.0% belonged to the ST131 lineage, including 94/137 (68.6%) ST131 H30 isolates. An increasing yearly prevalence was observed for ESBL-EC (from 4.6% to 9.4%; p=0.11), but not for the ST131 H30 subclone, which peaked in 2015 and declined thereafter. Multiple previously unnoticed ESBL-EC outbreaks occurred in the LTCF. Since 2018, we noted the clonal expansion of a rare ST131 H89 subclone (O16:H5) harboring CTX-M-14 and CTX-M-24. No rebound effect was observed in ESBL-EC prevalence nor in the different subclones following discontinuation of contact precautions for ESBL-EC carriers since 2019.

Conclusion:

Clonal fluctuation was observed for ST131 H30 ESBL-EC with a current decline in prevalence. Surveillance should include the evolution of ST131 non-H30 subclones, which may spread in LTCFs. Our findings suggest that discontinuation of contact precautions for ESBL-EC carriers in LTCFs may be safely implemented, in support of European recommendations to limit ESBL-producing Enterobacteriaceae control measures in endemic settings to non-*E.coli*.

Introduction

The global spread of extended-spectrum beta-lactamase producing *Escherichia coli* (ESBL-EC) is driven by the emergence of successful clones such as *E.coli* ST131, particularly transmissible in long-term care facilities (LTCFs) [1,2]. For instance, between 1996 and 2014, an increase of ESBL-EC was noticed in French LTCFs, reflecting clonal spread, with a 18.1% prevalence of ST131 clones [3,4]. In Swiss nursing homes, the proportion of ESBL-EC increased from 5% to 22% between 2007 and 2017 [5].

The increasing prevalence of *E.coli* ST131 among LTCFs is mostly explained by the clonal expansion of emerging multi-resistant clades of ESBL-EC [6], responsible for silent clusters among residents in LTCFs [7], including the fluoroquinolone-resistant clades C1 (C1/H30-R) and C2 (C2/H30-Rx) [8]. The reasons behind this apparent success are still controverted, but recent genomic and proteomic studies suggest that an improved anaerobic metabolism, as well as other human colonization and virulence factors helped this clone outcompeting the gut commensal niche, [9–11] with consecutive prolonged colonization. [12] This lineage particularly fostered the community spread of CTX-M, by the maintenance of clade-restricted MDR plasmids. [13] A nested cohort study of a large clinical trial recently observed the dominance of C1/H30-

R ESBL-EC in participating European LTCFs [14]. In that study, 49% (16/33) of all ESBL-EC ST131 carriers in Geneva were positive for C1/H30-R, compared to 20-39% in the 3 other centers outside Switzerland.

Considering the excess mortality and hospital stay associated with third-generation-cephalosporin-resistant *E.coli* [15,16], the epidemic potential of these ESBL-EC clades represents an infection control challenge in LTCFs, in particular in institutions without contact precautions for ESBL-EC carriers [17]. Effectively, many LTCFs around the world have discontinued contact precautions for ESBL-EC carriers, in light of recent studies on low nosocomial ESBL-EC transmission rates and endemic community carriage [17].

Specific aims

In our university-affiliated LTCF, yearly prevalence surveys were conducted from 2010 to 2020 as routine surveillance strategy to monitor the epidemiology of ESBL-producing Enterobacterales (ESBL-PE). In the present study, we sought to (i) characterize the temporal trends in the prevalence of ESBL-EC subclones among LTCF residents; (ii) combine epidemiological information with sequencing approaches to estimate the epidemic potential of emergent ESBL-EC subclones; and (iii), determine a potential rebound effect after de-implementation of contact precautions for carriers of these subclones.

Methods

Design and setting

This 11-year retrospective study was constituted by yearly prevalence surveys from 2010 through 2020, performed during January-February of each year, among all LCTF residents. Eight long-term care wards from a

same geographical site, representing 216 beds were included. From 2018 onwards, we added four long-term care wards from a second site, representing 73 beds.

Outcomes

The primary outcome included the overall prevalence of ESBL-EC carriage and abundance of different subclones across years, defined as the total number of positive cases per 100 screened residents. Secondary outcomes included the overall prevalence of ESBL-PE, the number of clusters (i.e. at least two residents sharing a ST131 H30, ST131 non-H30, and non-ST131 strain in the same ward the same year), the prevalence of subclones in the wards concerned by these clusters, and the proportion of clonally related strains among these clusters. Clonal relatedness was defined based on genomes, using a threshold in the pairwise distance of ≤10 SNP differences, as suggested elsewhere [18].

Infection control practices

In addition to standard precautions, until December 2018, all identified ESBL-PE carriers were placed under contact precautions, including gloves, hydrophobic coats, and, whenever possible, isolation in single-bed rooms. Contact precautions were abandoned for ESBL-EC from January 2019 onwards, with simultaneous reinforcement of standard precautions using routine observation and feedback from infection control nurses, in particular hand hygiene.

Health-related data

Epidemiological information was prospectively collected for each participant during the surveys, including ward location, admission date, date of sampling, previous positive cultures, age, and gender. We collected yearly hand hygiene adherence of healthcare workers in LTCFs from 2014 to 2020 according to WHO methods, as well as the length of

stay of all residents in the concerned wards during January and February, from 2010 to 2020.

Microbiological methods

Rectal swabs (E-swab, Copan) or stool cultures were collected for all participants, and processed using selective chromogenic agar (ChromID ESBL; bioMérieux). All colonies that met the expected chromogenic features provided in the manufacturers' specifications were identified by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (Bruker Daltonics, Bremen, Germany) and the antibiotic susceptibility profiles of each isolate was determined by the disk diffusion method using EUCAST breakpoints and recommendations [19]. Double-disk synergy tests (DDST20 and DDST30) were used for ESBL confirmation, ensuring a high sensitivity and specificity for ESBL-PE detection [20]. Assessment by ESBL + AmpC Screen Kit 98008 (Rosco Diagnostica, Danemark) was also performed to identify the partially de-repressed AmpC whenever the results of the DDST20 and cefoxitin tests were not conclusive.

Molecular typing

Allelic discrimination qPCR assays were performed on all newly detected ESBL-EC to ascertain ST131 lineages and H30 subtypes. For known carriers, we only retained the first ESBL-EC strain if isolated in the prior 12 months. Five single nucleotide polymorphism assays targeting specific positions in 2 genes used for MLST and constituting a unique signature of ST131 were selected and validated against a collection of >90 sequenced strains from highly diverse genetic backgrounds, as previously described [21]. The 6th assay was created from an existing *in silico* PCR and targets H30 through a coding point mutation in *FimH* sequence. Subclades ST131 H30 were then defined according to fluoroquinolone resistance (C1/H30-R) and additional presence of the *bla* gene CTX-M-15 (C2/H30-Rx).

Sequencing and assembly

Candidate strains for sequencing included ST131 H30 strains observed in large clusters since 2010 (with at least 4 positive cases from the same ward), and in all clusters since 2018 (with at least 2 positive ward mates). Moreover, all non-ST131 and ST131 non-H30 isolates from 2018 onwards were sequenced. Only the first isolate per patient and one morphotype per plate were considered for typing and sequencing. Purified genomic DNA (DNeasy, Qiagen) of selected isolates was sequenced using Illumina HiSeq2500 device using 100 base pairs (bp) paired-end reads and bar codes strategy according to the Nextera XT kit (Illumina), following the manufacturer's recommendations. Read quality was assessed with the Fastqc program (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and filtered using the FastqMcf program (Ea-utils; **Erreur ! Référence de lien hypertexte non valide.**). Genome assembly was performed using Spades assembler v 3.12.0. Assembled genomes were submitted individually to the Center for Genomic Epidemiology (https://cge.cbs.dtu.dk/) for confirmation of serotypes by using FimTyper 1.0 and SerotypeFinder 2.0.

Core genome multi-locus sequence typing target genes

The task template "E. coli cgMLST v1.0" was used in a multi-locus sequence typing (cgMLST) scheme with Ridom SeqSphere+ software version 5 (Ridom GmbH, Germany) using default settings. The final cgMLST scheme consisted of 2'513 genes covering roughly 45% of the genomic sequence of E. coli. From each isolate, the complete sequence of each gene was analyzed according to the cgMLST scheme and a numerical allele type was assigned to that given locus. The allelic profile was therefore determined by combining alleles of all cgMLST loci for each strain. A minimum spanning tree (MST) was inferred by neighbor joining

method on the allelic profiles. The remaining genes were used for pairwise-comparisons.

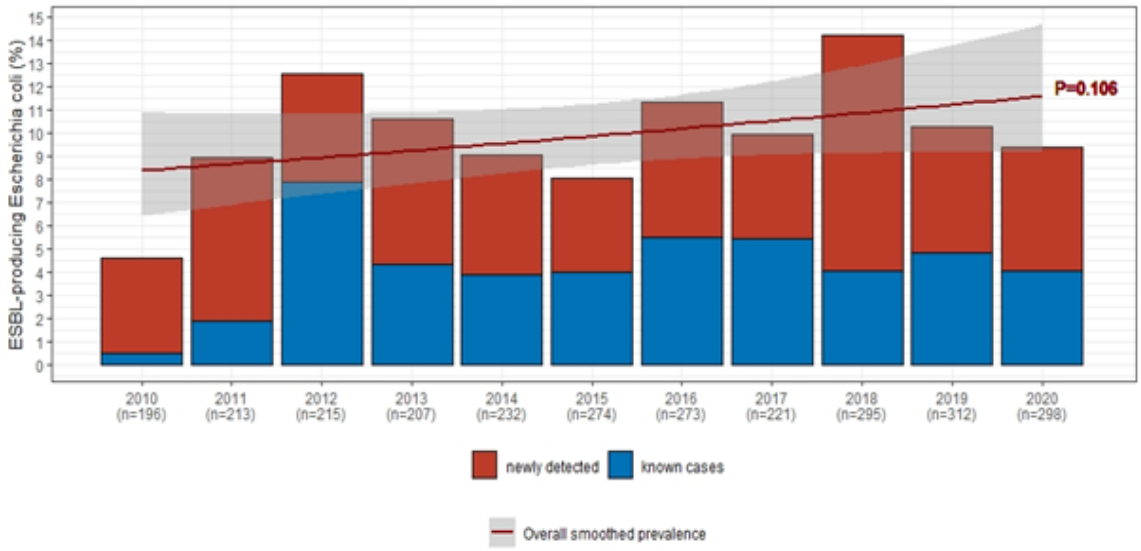
Statistical methods

Proportions were compared using χ^2 tests, or two-tailed Fisher's exact test when appropriate. The prevalence curve was segmented based on seeming inflection points for statistical comparison, as defined elsewhere [22]. A chi-square test for linear trend across these segments assessed prevalence shifts over time [23]. Genetic diversity was estimated using the number of ST divided by the number of strains sequenced. All analyses were conducted using R.4.0, including the package "lme4".

Results

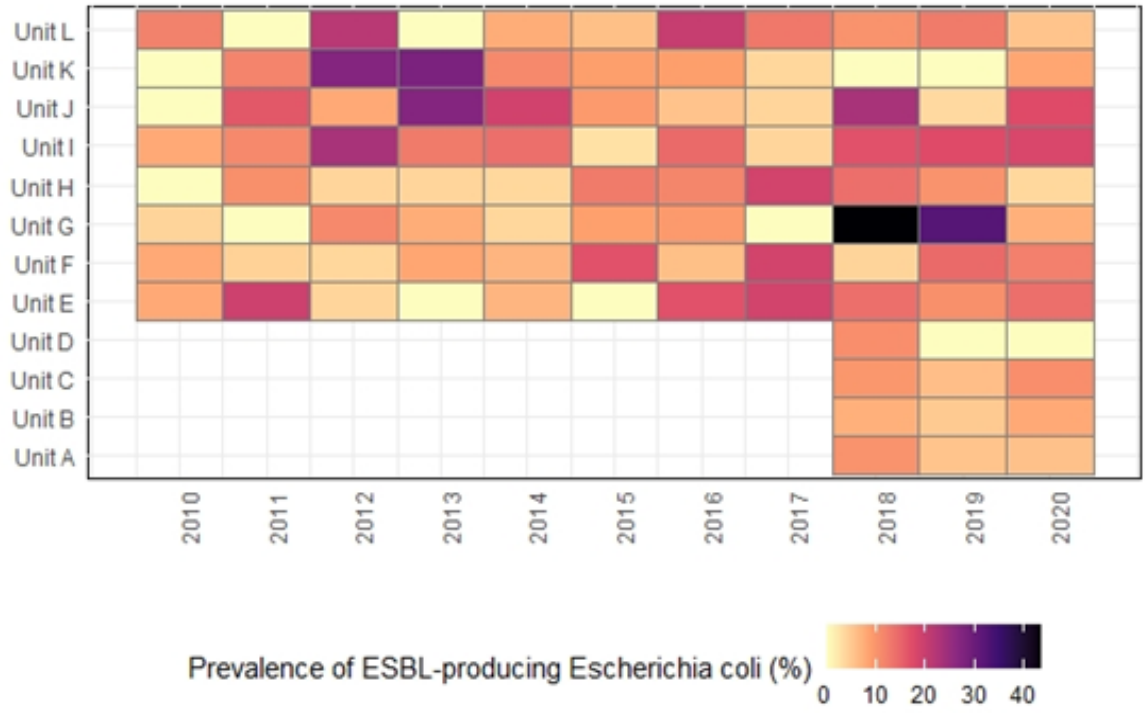
From January 2010 through February 2020, 11 yearly cross-sectional surveys of ESBL-PE carriage included 2'403 LTCF residents, with a median age of 83 years (IQR 75-89), and 61.4% of women. Yearly hand hygiene adherence improved from 72% to 77% from 2016 to 2020 (Suppl. Figure 1). The median length of stay of patients hospitalized in January and February from 2010 to 2020 decreased from 138.0 days (IQR 60.9-321.0) to 33.8 days (18.0-74.4.0). The total prevalence of any ESBL-PE carriage was 13.3% (n=319) and doubled from 7.1% to 13.8% over 10 years (p=0.04). Among ESBL-PE positive patients, 79.0% (n=252) and 18.8% (n=60) were respectively colonized with *E. coli* and *K. pneumoniae*. Over the study period, ESBL-EC prevalence increased from 4.6% to 9.4% (p=0.11), with a peak of 14.2% in 2018 (Fig. 1).

Fig 1. Prevalence of ESBL-producing Escherichia coli carriage among all residents of a university-affiliated long-term care facility from 2010 to 2020, before and after de-implementation of contact precautions in January 2019, and stratified between previously known and newly detected carriers.



We observed an increase of prevalent (previously known) cases from 11.1% to 43.0% of ESBL-EC from 2010 to 2020, with a stable proportion of incident (newly identified) cases. Of note, this increase was partly driven by nosocomial clusters throughout multiple wards in 2012, 2013, 2018, and 2019 (Fig. 2).

Fig 2. Yearly prevalence and clustering of ESBL-producing Escherichia coli carriers within 12 wards of a university-affiliated long-term care facility, Geneva (2010 to 2020). From 2018 onwards, 4 long-term care wards were added from a separate facility.



Overall, 58.0% (137/236) of typed ESBL-EC isolates belonged to the ST131 lineage, with 68.6% (94/137) positive for ST131 H30. The prevalence of this subclone remained stable until 2015 (Figure 3A), with a subsequent downward slope deflection from 2015 to 2020 (76.5% to 33.3%, $p<0.001$). No rebound effect was recorded neither for ESBL-EC, nor specifically for ESBL-EC ST131 H30 following de-implementation of contact precautions for ESBL-EC carriers in January 2019. In contrast, we observed an increase of ST131 non-H30 subtypes from 2016 to 2020 ($p=0.04$), which peaked in 2018 (Fig. 3A). In total, 82 of 236 (34.7%) typed ESBL-EC were sequenced, including 11 ST131 H30 strains from large nosocomial clusters in 2010-2017, 10 ST131 H30 strains from clusters in 2018-2020, as well as 24 ST131 non-H30 and 37 non-ST131

strains, isolated since 2018 (Suppl. Table 1). Among ST131 H30 isolates, 12 belonged to the clade C2/H30-Rx (57.1%), and 8 to the clade C1/H30-R (38.1%; Figure 3B).

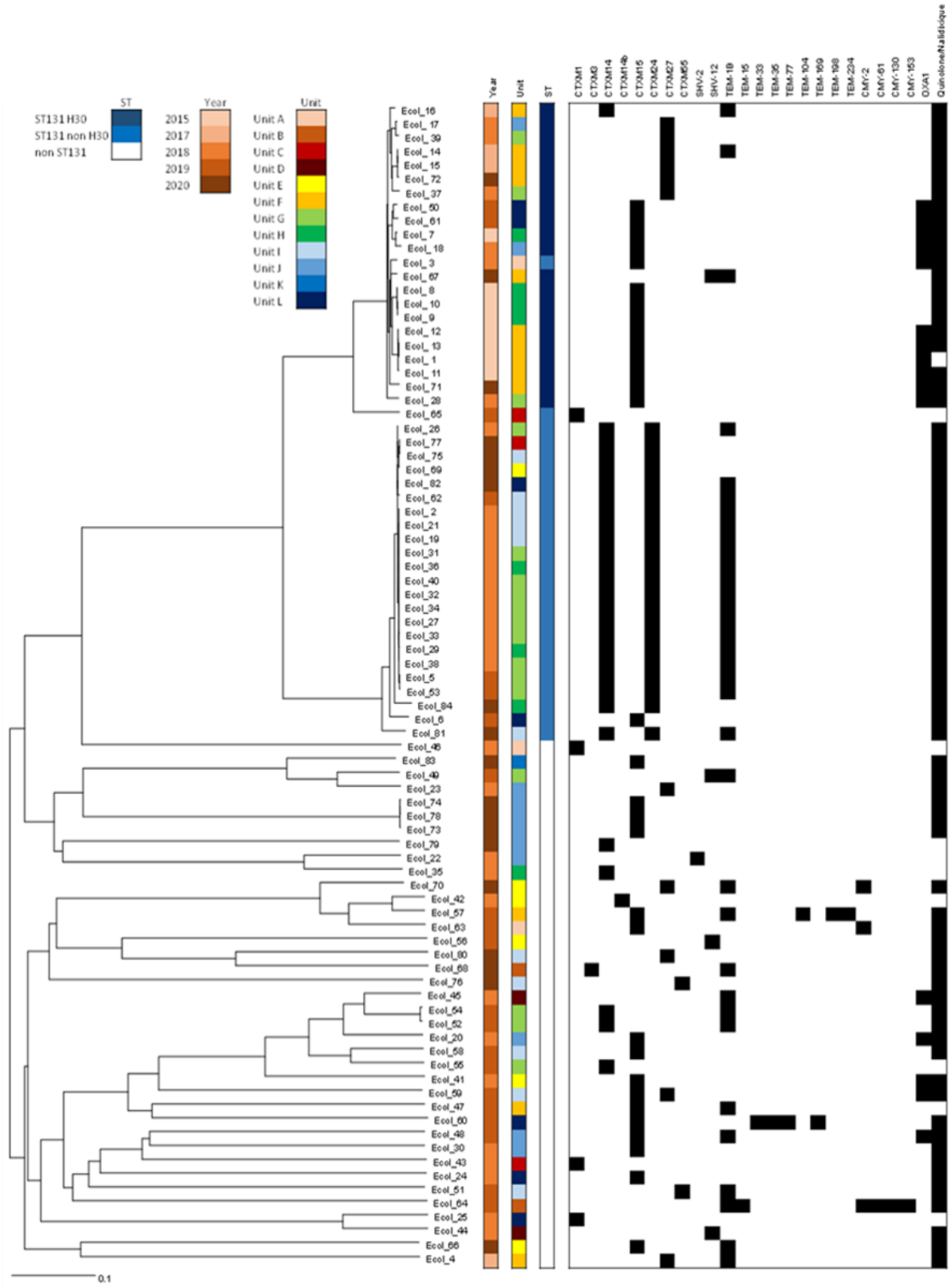
Fig 3. Prevalence of the different subclones among typed ESBL-producing Escherichia coli (ESBL-EC), and number of clades identified among sequenced ESBL-EC from participants of a university-affiliated long-term care facility from 2010 to 2020. (A) Subclones of typed ESBL-EC. Test for linear trends over segmented or continuous periods are indicated for the three subclones. Number of isolates per year are shown below the x-axis. (B) Subclones & clades of sequenced ESBL-EC ST131 since 2015.

Of note, whereas the majority of C2/H30-Rx strains (58.3%) were detected in 2015, C1/H30-R strains were only detected from 2017 onwards. Among ST131 non-H30, we observed the emergence and expansion of 22 (91.7%) isolates belonging to the ST131 H89 strain (O16:H5) associated with both CTX-M-14 and CTX-M-24. Among the 37

non-ST131 isolates, 31 different sequence types were identified, precluding possible monoclonal spread. Among ST131 H30, the most common resistance genes were CTXM-15 (72.2%), OXA-1 (55.6%), and CTXM-27 (22.2%). Among ST131 non H30, the most common resistance genes were CTXM-14 (91.7%), CTXM-24 (91.7%), and TEM-1B (75.0%). Among non-ST131, the most common resistance genes were CTXM-15 (43.2%), TEM-1B (32.4%), OXA-1 (13.5%), and CTXM-14 (13.5%).

When considering epidemiological information from 2010 to 2020, we observed 27 nosocomial clusters of patients positive for ESBL-EC ST131 H30. Almost all (20/21) ESBL-EC ST131 H30 strains available for sequencing were genotypically related (Fig. 4). C2/H30-Rx strains dominated in 2015, while C1/H30-R was present in more recent clusters. Sixteen of these 21 (76%) strains were isolated from 2 wards (unit F and H) between 2015 and 2020. Twenty of 24 (83%) ST131 non-H30 strains available for sequencing were genotypically related and identified as ST131 H89 with the serotype O16:H5, which expressed the same CTX-M-14 and CTX-M-24 genes; We observed 5 clusters of patients positive for ST131 H89 in three wards (wards G, H, and I), with an attack rate of 12% (17 of 139 susceptible patients, Fig. 3). Finally, only 18% (5/27) of sequenced non-ST131 strains were genotypically related (Fig. 4).

Fig 4. Dendrogram of sequenced ESBL-producing Escherichia coli, with epidemiological information and molecular data on ESBL genes; PCRH30 represent the results from the multi-array PCR, with non-ST131, ST131 H30, and ST131 non-H30.



Discussion

The findings of these 11 yearly cross-sectional surveys support five main conclusions: (1) ESBL-EC prevalence increased over time in this

university-affiliated LTCF, mainly driven by an increased proportion of previously known carriers; (2) after 2015, a decreasing prevalence of ST131 H30 subclones was observed over time, despite small localized outbreaks; (3) clonal expansion of ST131 H89 (O16:H5) subclones occurred since 2018, driven by multiple silent outbreaks; (4) no emerging non-ST131 clone was observed; and (5) no rebound effect in ESBL-EC or specific subclones was observed following discontinuation of contact precautions, though longer follow-up periods are needed to validate this finding.

LTCFs are well-known reservoirs for multiresistant ESBL-EC clones, with specific patient- and care-related exposures facilitating the spread of certain clades, including vulnerable and dependent patients with prolonged lengths of stay [24–29], as well as recognized challenges in implementing infection control measures [24,28]. Many outbreaks report silent transmission of ESBL-EC ST131 in LTCF, especially belonging to the clade C2 (C2/H30-Rx-CTX-M-15) [1,2,6]. The rapid clonal expansion of this C2 clade through nosocomial outbreaks in LTCFs has already been observed to displace preexisting E.coli clades [6]. Thus, clonal fluctuance has been a recognized phenomenon with emergence and decline of temporarily successful clones. The persistence of certain E. coli clones, sporadically carrying carbapenemases genes, warrants a careful surveillance. [30]

Until now, few studies have reported nosocomial outbreaks associated with E.coli ST131 non-H30 clades. Population genomics on 4’071 globally sources genomes observed a dominance of the clade C, co-circulating worldwide at stable frequencies [31]. In 2018, a single Spanish LTCF of 300 residents observed only 6 ST131 non-H30 associated with CTX-M-14 on 55 typed ESBL-EC isolates [32]. Our study observed that neither E.coli C2/H30-Rx, nor C1/H30-R, seem to drive the recent changing epidemiology of ESBL-EC in our LTCF, but rather a ST131 H89 harboring

CTX-M-14 and CTX-M-27. This strain was sub-typed based on its fimH typing region, which is closely related and often associated to the H41 group of E.coli ST131 (1 SNP difference) [33–35]. To the best of our knowledge, there has been no outbreak report of this ST131 H89 E.coli subclone, which is anecdotally reported in population genomics [31,35,36].

The findings of this study are also in line with currently available evidence, which supports the discontinuation of contact precautions for ESBL-EC carriers, as suggested by European recommendations to limit ESBL-PE control measures in endemic settings to non-E.coli ESBL-PE [17]. Clonal outbreaks already occurred before discontinuation of contact precautions, and were not catalyzed by this decision. The observed recent clusters might not be an effect from lack of strict contact precautions, but rather direct consequences of the LTCF infrastructure, among other factors related to this specific setting [28,37].

Though this study includes a large sample size and long-term surveillance data, we acknowledge the presence of several limitations. First, we could not quantify transmission and acquisition events due to the study design. Second, we acknowledge a potential bias in the selection of E.coli strains sequenced. Third, generalizability of our findings is impacted by the unicentric approach. Fourth, potential lack of genomic discrimination between highly similar E.coli clades was not possible due to the sequencing methods used (short reads). Fourth, the decreased length of stay could impact ESBL-EC prevalence by lowering the probability of ESBL-EC acquisition and the proportion of known carriers. However, the acquisition risk in relation to the length of stay has been observed to be similar between ST131 and non-ST131 E.coli, and did not differ between 6 and 8 months of stay in LTCF. [12] Furthermore, ESBL-EC prevalence appears similar between LTCFs and among elderly in community. [38]

For these reasons, the decreasing length of stay probably did not influence ESBL-EC prevalence.

Conclusions

The changing ESBL-EC epidemiology, emergence of novel clones, and related clusters in LTCF, though not impacted by discontinuation of contact precautions, should be monitored by a comprehensive screening and surveillance strategy.

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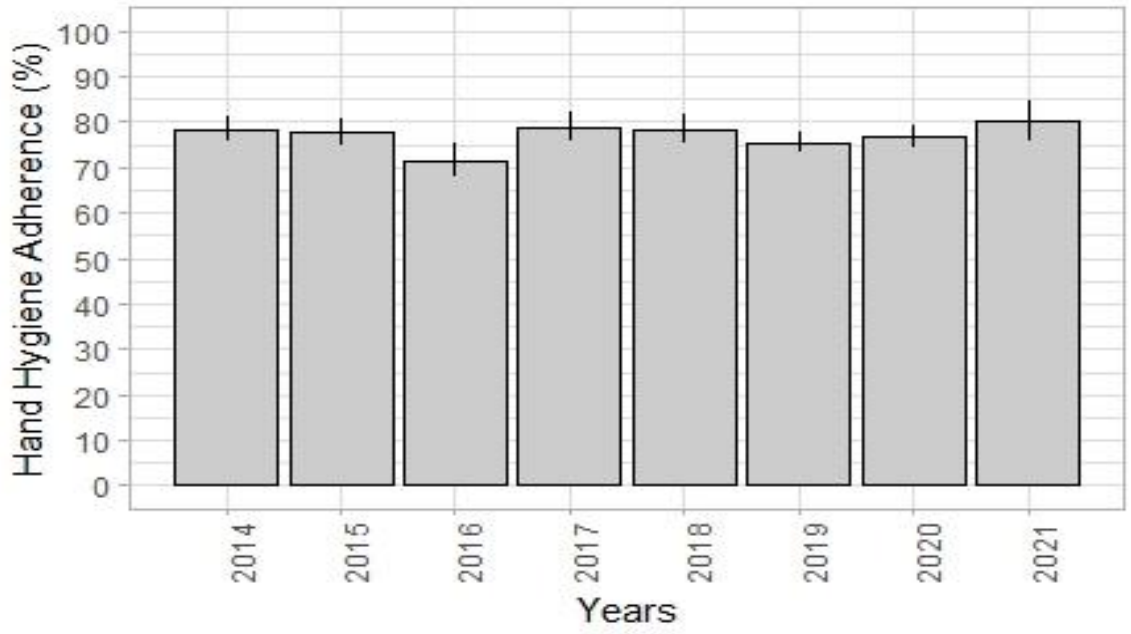
Supplementary Appendix

Supplementary Figures

Figure S1. Hand Hygiene Adherence of healthcare workers in Long Term Care Facilities from 2014 to 2021



Supplementary Tables

Table S1. Epidemiologic and genotypic characteristics of sequenced ESBL-producing Escherichia coli

Strain	Unite	Year	ST [a]	PCR H30	fimH	Serotype	ESBL	Quinolone/Nalidixique
MR1	F	2015	131	1			CTX-M-15;OXA-1	
MR2	I	2018	131	0	89	H5O16	CTX-M-14;CTX-M-24;TEM-1B	gyrA (p.S83L)
MR5	G	2019	131	0	89	H5O16	CTX-M-14;CTX-M-24;TEM-1B	gyrA (p.S83L)
MR6	L	2019	131	0	41		CTX-M-15	gyrA (p.S83L)
MR7	H	2015	131	1			CTX-M-15;OXA-1	gyrA (p.S83L), gyrA (p.D87N)
MR8	H	2015	131	1			CTX-M-15	gyrA (p.D87N), gyrA (p.S83L)
MR9	H	2015	131	1			CTX-M-15	gyrA (p.D87N), gyrA (p.S83L)
MR10	H	2015	131	1			CTX-M-15	gyrA (p.S83L), gyrA (p.D87N)
MR11	F	2015	131	1			CTX-M-15;OXA-1	gyrA (p.S83L), gyrA (p.D87N)
MR12	F	2015	131	1			CTX-M-15;OXA-1	gyrA (p.S83L), gyrA (p.D87N)
MR13	F	2015	131	1			CTX-M-15;OXA-1	gyrA (p.D87N), gyrA (p.S83L)
MR14	F	2017	131	1			CTX-M-27;TEM-1B	gyrA (p.S83L), gyrA (p.D87N)
MR15	F	2017	131	1			CTX-M-27	gyrA (p.S83L), gyrA (p.D87N)
MR16	F	2017	131	1			CTX-M-14;TEM-1B	gyrA (p.D87N), gyrA (p.S83L)
MR17	J	2018	131	1			CTX-M-27	gyrA (p.S83L), gyrA (p.D87N)
MR18	J	2018	131	1			CTX-M-15;OXA-1	gyrA (p.D87N), gyrA (p.S83L)
MR19	I	2018	131	0	89	H5O16	CTX-M-14;CTX-M-24;TEM-1B	gyrA (p.S83L)
MR20	J	2018	10	0	435		CTX-M-15;OXA-1	gyrA (p.D87N), gyrA (p.S83L)

MR21	I	*2018*	*131*	*0*	*89*	*H5O16*	*CTX-M-14;CTX-M-24;TEM-1B*	*gyrA (p.S83L)*
MR22	J	*2018*	*73*	*0*	*10*		*SHV-2*	
MR23	J	*2018*	*1193*	*0*	*64*		*CTX-M-27*	*gyrA (p.D87N), gyrA (p.S83L)*
MR24	L	*2018*	*410*	*0*	*24*		*CTX-M-15*	*gyrA (p.D87N), gyrA (p.S83L)*
MR25	L	*2018*	*5926*	*0*	*158*		*CTX-M-1*	
MR26	G	*2018*	*131*	*0*	*89*	*H5O16*	*CTX-M-14;CTX-M-24;TEM-1B*	*gyrA (p.S83L)*
MR27	G	*2018*	*131*	*0*	*89*	*H5O16*	*CTX-M-14;CTX-M-24;TEM-1B*	*gyrA (p.S83L)*
MR28	G	*2018*	*131*	*1*			*CTX-M-15;OXA-1*	*gyrA (p.S83L), gyrA (p.D87N)/aac(6')-Ib-cr (aac(6')-Ib-cr_DQ303918) gyrA (p.S83L)*
MR29	H	*2018*	*131*	*0*	*89*	*H5O16*	*CTX-M-14;CTX-M-24;TEM-1B*	*gyrA (p.S83L)*
MR30	J	*2018*	*1431*	*0*	*32*		*CTX-M-15*	*gyrA (p.D87N), gyrA (p.S83L)*
MR31	G	*2018*	*131*	*0*	*89*	*H5O16*	*CTX-M-14;CTX-M-24;TEM-1B*	*gyrA (p.S83L)*
MR32	G	*2018*	*131*	*0*	*89*	*H5O16*	*CTX-M-14;CTX-M-24;TEM-1B*	*gyrA (p.S83L)*
MR33	G	*2018*	*131*	*0*	*89*	*H5O16*	*CTX-M-14;CTX-M-24;TEM-1B*	*gyrA (p.S83L)*
MR34	G	*2018*	*131*	*0*	*89*	*H5O16*	*CTX-M-14;CTX-M-24;TEM-1B*	*gyrA (p.S83L)*
MR35	H	*2018*	*104*	*0*	*2*		*CTX-M-14*	

MR36	H	*2018*	*131*	*0*	*89*	*H5O16*	*CTX-M-14;CTX-M-24;TEM-1B*	*gyrA (p.S83L)*
MR37	G	*2018*	*131*	*1*			*CTX-M-27*	*gyrA (p.D87N), gyrA (p.S83L)*
MR38	G	*2018*	*131*	*0*	*89*	*H5O16*	*CTX-M-14;CTX-M-24;TEM-1B*	*gyrA (p.S83L)*
MR39	G	*2018*	*131*	*1*			*CTX-M-27*	*gyrA (p.D87N), gyrA (p.S83L)*
MR40	G	*2018*	*131*	*0*	*89*	*H5O16*	*CTX-M-14;CTX-M-24;TEM-1B*	*gyrA (p.S83L)*
MR41	E	*2018*	*617*	*0*	*29*		*CTX-M-15;OXA-1*	*gyrA (p.S83L), gyrA (p.D87N)*
MR42	E	*2018*	*38*	*0*	*ND*		*CTX-M-14b*	
MR43	C	*2018*	*224*	*0*	*61*		*CTX-M-1*	*gyrA (p.S83L), gyrA (p.D87N)*
MR44	D	*2018*	*57*	*0*	*27*		*SHV-12*	*gyrA (p.D87N), gyrA (p.S83L)*
MR45	D	*2018*	*10*	*0*	*54*		*TEM-1B;OXA-1*	*gyrA (p.D87N), gyrA (p.S83L)*
MR46	A	*2018*	*538*	*0*	*46*		*CTX-M-1*	
MR47	F	*2019*	*8149*	*0*	*ND*		*CTX-M-15;TEM-1B*	
MR48	J	*2019*	*191*	*0*	*38*		*CTX-M-15;TEM-1B;OXA-1*	*qnrB1 (qnrB1_DQ351241), aac(6')-Ib-cr (aac(6')-Ib-cr_DQ303918)*
MR49	G	*2019*	*14*	*0*	*27*		*SHV-12;TEM-1B*	*gyrA (p.S83L)*
MR50	L	*2019*	*131*	*1*			*CTX-M-15;OXA-1*	*aac(6')-Ib-cr (aac(6')-Ib-cr_DQ303918) gyrA (p.S83L)*
MR51	I	*2019*	*6448*	*0*	*60*		*CTX-M-55;TEM-1B*	*gyrA (p.S83L), gyrA (p.D87N)*
MR52	G	*2019*	*167*	*0*	*ND*		*CTX-M-14;TEM-1B*	*gyrA (p.D87N), gyrA (p.S83L)*

MR53	G	*2019*	*131*	*0*	*89*	*H5O16*	*CTX-M-14;CTX-M-24;TEM-1B*	*gyrA (p.S83L)*
MR54	G	*2019*	*167*	*0*	*ND*		*CTX-M-14;TEM-1B*	*gyrA (p.D87N), gyrA (p.S83L)*
MR55	G	*2019*	*10*	*0*	*27*		*CTX-M-14*	
MR56	E	*2019*	*925*	*0*	*54*		*SHV-12*	*qnrS1 (qnrS1_AB187515)*
MR57	F	*2019*	*38*	*0*	*5*		*CTX-M-15;TEM-1B;TEM-104;TEM-198;TEM-234*	*gyrA (p.S83L)*
MR58	I	*2019*	*3877*	*0*	*27*		*CTX-M-15*	*qnrS1 (qnrS1_AB187515)*
MR59	I	*2019*	*226*	*0*	*41*		*CTX-M-15;CTX-M-27;OXA-1*	*gyrA (p.S83L), gyrA (p.D87N)/qnrS13 (qnrS13_LUYD01000008) gyrA (p.S83L)*
MR60	L	*2019*	*46*	*0*	*34*		*CTX-M-15;TEM-33;TEM-35;TEM-77;TEM-169*	*qnrS1 (qnrS1_AB187515)*
MR61	L	*2019*	*131*	*1*			*CTX-M-15;OXA-1*	*aac(6')-Ib-cr (aac(6')-Ib-cr_DQ303918) gyrA (p.S83L)*
MR62	I	*2019*	*131*	*0*	*89*	*H5O16*	*CTX-M-14;CTX-M-24;TEM-1B*	*gyrA (p.S83L)*
MR63	A	*2019*	*3268*	*0*	*54*		*CTX-M-15;CMY-2*	*qnrS1 (qnrS1_AB187515)*
MR64	B	*2019*	*120*	*0*	*237*		*TEM-1B;TEM-15;CMY-2;CMY-61;CMY-130;CMY-153*	*qnrB19 (qnrB19_EU432277)*
MR65	C	*2019*	*131*	*0*	*22*	*H4O25*	*CTX-M-1*	
MR66	E	*2020*	*1722*	*0*	*153*		*CTX-M-15;TEM-1B*	*qnrS1 (qnrS1_AB187515)*
MR67	F	*2020*	*131*	*1*			*SHV-12;TEM-1B*	*gyrA (p.S83L), gyrA (p.D87N)*
MR68	B	*2020*	*1380*	*0*	*47*		*CTX-M-3;TEM-1B*	*gyrA (p.S83L)*

MR69	E	*2020*	*131*	*0*	*89*	*H5O16*	*CTX-M-14;CTX-M-24*	*gyrA (p.S83L)*
MR70	E	*2020*	*5150*	*0*	*65*		*CTX-M-27;TEM-1B;CMY-2*	*gyrA (p.S83L)*
MR71	F	*2020*	*131*	*1*			*CTX-M-15;OXA-1*	*gyrA (p.S83L), gyrA (p.D87N) - aac(6')-Ib-cr (aac(6')-Ib-cr_DQ303918) gyrA (p.S83L)*
MR72	F	*2020*	*131*	*1*			*CTX-M-27*	*gyrA (p.S83L), gyrA (p.D87N)*
MR73	J	*2020*	*636*	*0*	*ND*		*CTX-M-15*	*gyrA (p.S83L)*
MR74	J	*2020*	*636*	*0*	*ND*		*CTX-M-15*	*gyrA (p.S83L)*
MR75	I	*2020*	*131*	*0*	*89*	*H5O16*	*CTX-M-14;CTX-M-24*	*gyrA (p.S83L)*
MR76	I	*2020*	*349*	*0*	*54*		*CTX-M-55*	-
MR77	C	*2020*	*131*	*0*	*89*	*H5O16*	*CTX-M-14;CTX-M-24*	*gyrA (p.S83L)*
MR78	J	*2020*	*636*	*0*	*ND*		*CTX-M-15*	*gyrA (p.S83L)*
MR79	J	*2020*	*681*	*0*	*3*		*CTX-M-14*	
MR80	I	*2020*	*69*	*0*	*27*		*CTX-M-27*	*gyrA (p.S83L)*
MR81	I	*2020*	*131*	*0*	*89*	*HXO16*	*CTX-M-14;CTX-M-24;TEM-1B*	*gyrA;;bvzc (p.S83L)*
MR82	L	*2020*	*131*	*0*	*89*	*H5O16*	*CTX-M-14;CTX-M-24;TEM-1B*	*gyrA (p.S83L)*
MR83	K	*2020*	*1446*	*0*	*30*		*CTX-M-15*	*qnrS1 (qnrS1_AB187515)*
MR84	H	*2020*	*131*	*0*	*89*	*H5O16*	*CTX-M-14;CTX-M-24*	*gyrA (p.S83L)*

Footnote to Suppl. Table 1.

a Sequence types were determined based on the allelic discrimination qPCR assays described in the methods to ascertain ST131 lineages, and based on MLST for negative ST131 results.

Part 2) Household carriage and acquisition of extended-spectrum β-lactamase-producing Enterobacteriaceae: A systematic review

A similar version of this chapter was published under the following reference:

Martischang R, Riccio ME, Abbas M, Stewardson AJ, Kluytmans JAJW, Harbarth S. Household carriage and acquisition of extended-spectrum b-lactamase-producing Enterobacteriaceae: A systematic review. Infect Control Hosp Epidemiol. 2020;41(3):286-294. DOI: 10.1017/ice.2019.336.

Abstract

Objective:

While the epidemiology of ESBL-producing Enterobacteriaceae (ESBL-PE) has been extensively studied in hospitals, data on community transmission is scarce. We conducted a systematic review to assess ESBL-PE co-carriage and acquisition in households.

Methods:

A systematic literature search was conducted to retrieve cross-sectional or cohort studies published between 1990 and 2018 evaluating co-carriage proportions and/or acquisition rates of ESBL-PE among household members, without language restriction. We excluded studies focusing on animal-to-human transmission or non-household settings. The main outcomes were ESBL-PE co-carriage proportions and acquisition rates, stratified according to phenotypic or genotypic assessment of strain relatedness. Co-carriage proportions of clonally-related ESBL-PE were transformed via the double-arcsine method and pooled using a random-effects model. Potential biases were assessed manually.

Results:

We included 13 studies. Among 863 household members of ESBL-PE positive index cases, prevalence of ESBL-PE co-carriage ranged from 8% to 37%. Overall, 12% (95%CI: 8-16%) of subjects had a clonally-related strain. Those proportions were higher for Klebsiella pneumoniae (20-25%) compared to Escherichia coli (10-20%). Acquisition rates of clonally-related ESBL-PE among 180 initially ESBL-PE free household members of a previously identified carrier ranged between 1.56 - 2.03 events per 1000 person-weeks of follow-up. We identified multiple sources of bias and large heterogeneity (I^2: 70%) between studies.

Conclusions:

ESBL-PE household co-carriage is frequent, suggesting intra-familial acquisition. Further research is needed to evaluate the risk and control of ESBL-PE household transmission.

Introduction

The prevalence of extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-PE) in the general population has now reached endemic levels in most countries.(39) This is worrisome, since ESBL-PE are frequent causes of difficult-to-treat infections, with substantial health-economic burden.(40)

ESBL-PE may spread by transfer of bacteria or mobile genetic elements. Some biologically fit phylogenetic groups particularly drive the emergence and persistence of virulence traits and acquisition of ESBL-PE.(41) Persistence of ESBL-PE in the community might be further amplified by various risk factors such as antibiotic exposure,(42–45) previous hospitalization,(43,46) recurrent urinary tract infection,(43) travel activities,(46,47) having children attending daycare centers,(48) as well as chicken meat consumption.(49) Overcrowded households also appear to increase the risk of ESBL-PE carriage.(50) Furthermore, intra-household transmission may play an important but understudied role. Several studies have shown that antibiotic-susceptible and resistant *Escherichia coli* are transmitted between household members,(51) suggesting that both susceptible and resistant Enterobacteriaceae compete for niches within the gastrointestinal tract. This competitive balance is influenced by multiple factors including antibiotic exposure, which favors resistant Enterobacteriaceae and their intra-household transmission.(42)

Specific aims

Despite the potential relevance of ESBL-PE cross-transmission among household members on persistence and spread of ESBL-PE in the community, evidence on this topic is scarce. We therefore aimed to systematically review epidemiological studies on ESBL-PE co-carriage and acquisition among household members.

Methods

Data sources and search strategy

We searched the Cochrane Library, PubMed, Embase and CINAHL databases for observational studies published between January 1990 and June 2018, without language restriction. Systematic manual reference search was performed from eligible articles' bibliography. Duplicate studies with the same title and authors were automatically deleted by the «Distiller» SR software (Evidence Partners, Ottawa, Canada). Core search strings, assembled Boolean operators, included: « household OR community OR family OR outpatient » and « animal OR pet » for the study population; « extended-spectrum beta-lactamase OR lactamase OR cephalosporin OR beta-lactam resistance » for exposures; and « transmission OR carriage OR acquisition OR colonization OR microbiota OR molecular epidemiology » for outcomes. The full search strategies are available in the Supplementary Appendix. This study was conducted according to the MOOSE and PRISMA statements.(52)

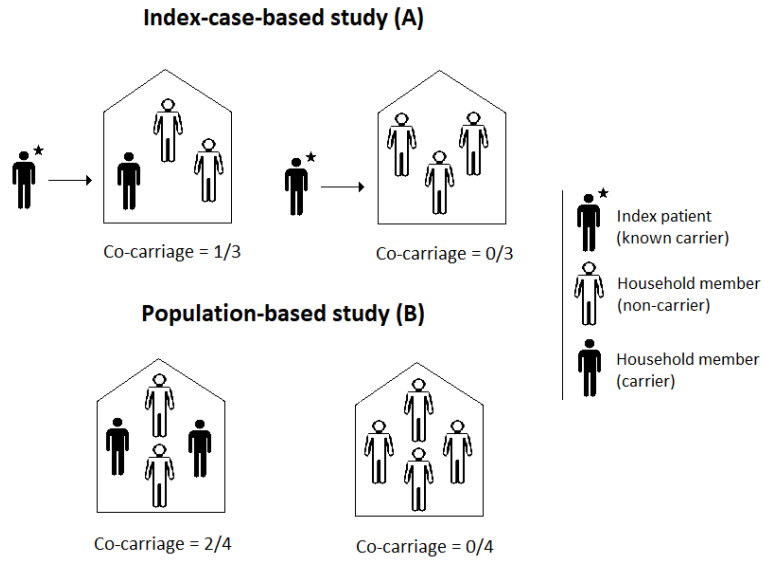
Selection criteria and definitions

This systematic review includes cohort or cross-sectional studies evaluating co-carriage proportions and acquisition rates of ESBL-PE in households, focusing mainly on intestinal carriage of E. coli and Klebsiella pneumoniae. ESBL-PE were defined phenotypically by presence of 3rd

generation cephalosporin resistance and a positive double-disk synergy test, and/or genotypically by an identified ESBL-PE resistance gene. Studies were eligible if they included isolates sampled from human subjects. Co-carriage was defined as simultaneous carriage by two or more household members of a related ESBL-PE strain at a certain point in time or during a pre-defined follow-up period. Acquisition was defined as newly identified carriage of a related strain in another household member who was previously ESBL-negative. Relatedness definition depended on the level of microbiological discrimination employed. Co-carriage and acquisition rates were stratified considering the level of microbiological discrimination: "closely-related" pathogens were phenotypically similar bacteria, sharing the same phenotypic or genotypic resistance profile; "clonally-related" pathogens were bacteria assessed for relatedness through genotyping methods.

Studies were stratified according to their sampling scheme. In index-case-based study (category A), recruitment of families derived from a previously identified ESBL-PE index-case. In population-based study (category B), household members were not recruited based on a previously known index case, but from the general population. In category A, co-carriage proportions were calculated as the number of household members of a colonized or infected index case simultaneously carrying a closely-related or clonally-related ESBL-PE, among the total number of household members (excluding the index case). In category B, all household members presenting simultaneous ESBL-PE-related carriage among the total number of household members were considered (Figure 1).

Figure 1. Examples for the evaluation of co-carriage estimates in studies based on their sampling schemes



We excluded single-household case reports, studies focusing on animal-to-animal or animal-to-human transmission only, as well as studies focusing on the environment (e.g. surface water) or non-household settings (e.g. child-care facilities). Studies focusing on international travelers, indigenous populations with a specific way of living, farms, or food-borne community outbreaks were excluded. Due to specific exposures and an extensive literature on the topic, studies on mother-to-newborn transmission were also excluded.

Study screening and data extraction

Title and abstract screening was done independently by two authors (R.M., M.E.R.). All discrepancies were solved by consensus, involving a third investigator (M.A.) if needed. Concordance was checked by Cohen's kappa coefficient. One author (R.M.) performed full-text screening and data extraction, with any uncertainties resolved by discussion with another author (M.E.R.). We extracted the following data: study characteristics (study dates, design, outcomes, follow-up), study population (characteristics of index cases and families, number of

household members, potential biases addressed) and microbiological methods. As primary outcome, ESBL-PE co-carriage proportions and acquisition rates were calculated based on available information. Preferably, co-carriage proportions of longitudinal studies were generated at baseline as a point-prevalence, to be able to compare them with cross-sectional studies. However, if no such information was available, co-carriage proportions of the overall follow-up period were reported as a period prevalence. Both study screening and data extraction were performed using standardized electronic forms through DistillerSR software. Potential clinical and microbiological confounders were specifically reported, both for index cases and their household members. Characteristics of household members, sampling methods, loss to follow-up, hospital stay, antibiotic exposure, travel activity, food intake, day-care centers and socio-economic status were considered as clinically relevant. The number of colonies analyzed per morphotype and the use of broth enrichment were considered as microbiologically relevant.

Statistical analysis

The main outcomes of interest were the proportion of co-carriage and rate of acquisition among household members, stratified by the study type, which was defined by its sampling scheme, and microbiological discrimination level (as detailed above). Co-carriage proportions of closely-related and clonally-related Enterobacteriaceae were compared. Co-carriage proportions of household members with clonally-related ESBL-PE from index-case based studies were pooled using meta and metafor packages.(53,54) Double-arcsine transformation was applied on raw proportions to estimate a normal distribution before pooling.(55) Transformed individual effect sizes were then pooled using a random-effects model to account for between-study variance. Heterogeneity among effect size was estimated using the Q test and I^2 test. Subgroup comparisons were performed to explore relationships and heterogeneities, by stratifying individual-based co-carriage among the

proportion of species isolated from index cases (> or <15% of K. pneumoniae). Potential publication bias or small-study effects were examined by funnel plot. All analyses were performed using the R open-source software environment, version 3.4.4 (R code available in the Appendix).

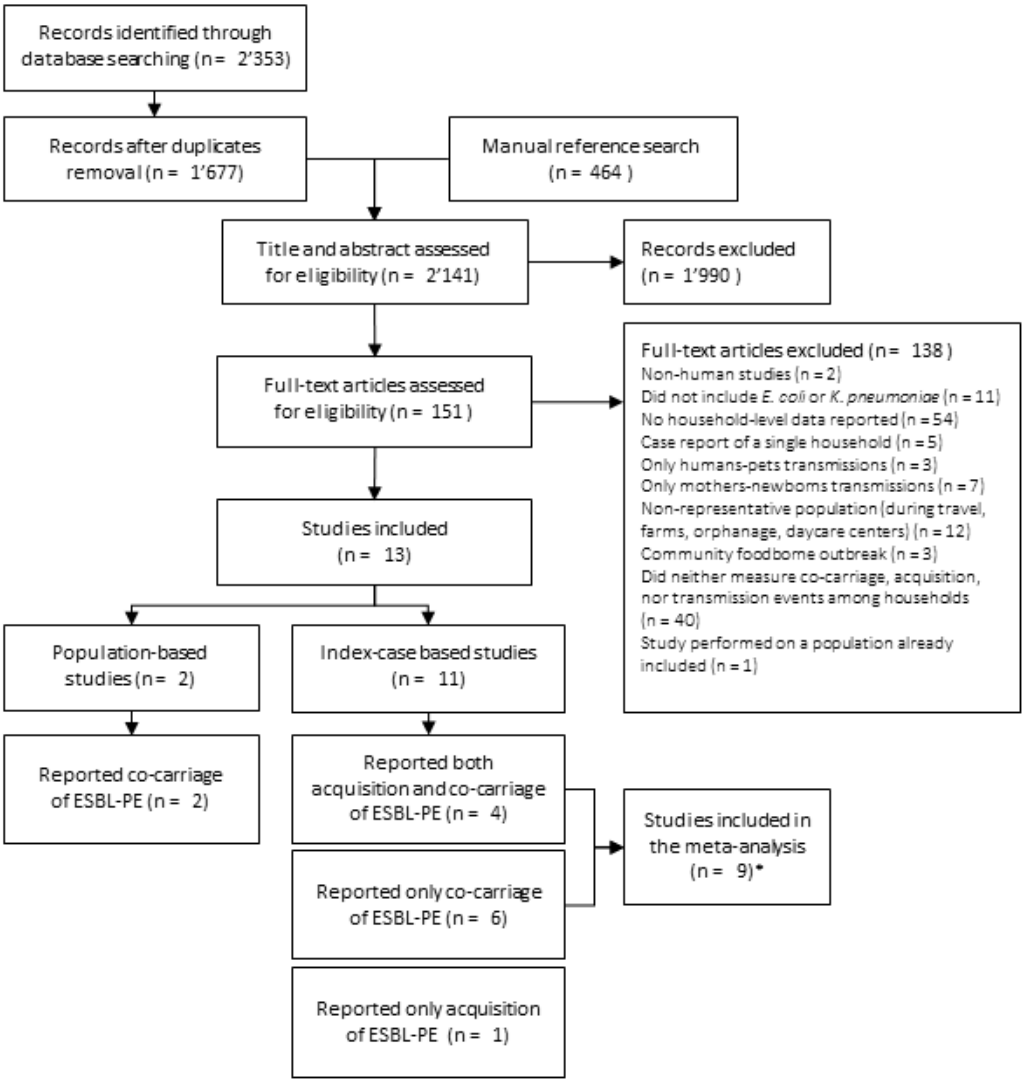
Results

Study selection and features of included studies

The literature search identified 2,353 articles. After duplicate removal, 2,141 articles were screened for eligibility. A total of 151 articles underwent full-text screening (Kappa, 0.80). Finally, 13 studies[42,56–67] were selected for data extraction and bias assessment (Figure 2). Two publications initially classified as population-based studies qualified as an index-case study, since we were able to extract household co-carriage and acquisition rates with at least one colonized member from the crude data.[42,60] Thus, sampling schemes were population-based and index-case-based for 2 and 11 studies, respectively. The 2 population-based studies were considered as cross-sectional,[59,63] and of the 11 index-case based studies, 7 were longitudinal cohort studies [42,58,62,64–67] and 4 were cross-sectional studies.[56,57,60,61] Two longitudinal studies were considered as nested cross-sectional studies for the purpose of our review, because after a first baseline sampling at home subsequent follow-up happened only in a hospital setting.[56,63] Co-carriage data were not collected for one index-case based study which only included previously negative household members.[62] Another index-case based study, only reporting co-carriage of closely-related bacteria,[42] was excluded from the meta-analysis, which focused only on those 9 studies with data on co-carriage of clonally reported pathogens. Acquisition rates were extracted and calculated from 5 of the 7 index-case based cohort

studies, excluding two studies with unknown ESBL-PE status of household members at baseline.(58,67)

Figure 2. Systematic review flow-chart detailing the study selection procedure



*Footnote to figure 2: *only studies evaluating co-carriage of clonally-related Extended-Spectrum β-lactamases Producing Enterobacteriaceae (ESBL-PE) were included in the meta-analysis*

Study population

The main characteristics of the included studies are displayed in Table 1. For index-case based studies, sample sizes ranged from 46 to 286 household members, and for population-based studies, from 225 to 753 household members. The 9 studies based on index cases defined them by being colonized[56,58,62,64,65] or infected[57,61] with ESBL-PE, or both.[66,67] The four population-based studies recruited household members from inpatient,[59,63] outpatient[42,59] or healthy community settings.[60] Of the 13 studies, 3 recruited an entire family[59,64,66] and 10 recruited a convenience sample of at least 2 household participants.[42,56–58,60–63,65,67]

1 *Table 1. Study population and characteristics of included studies*

Bibliography	Country	ESBL species isolated *(for the index cases if not specified)*	Follow up duration	Category of participants	ESBL-PE status at inclusion	Population size	Age (median, IQR)	Gender (Female, %)	Co-carriage considered
Cross-over population based studies without index cases									
Lo WU, et al. 2010 [21]	China	81% *E.coli*, 19% *K. pneumoniae (Among all participants)*	NA	First group of household members	Unknown	53	2 years (0.8-3)	21 (40%)	Closely related: CTXM-PE Clonally related: CTXM strain
				Second group of household members	Unknown	172	5 years (29 infants) (2.3-8) 35 years (143 adults) (31-43)	104 (60%)	
Kurz MS, et al. 2017 [25]	Rwanda	48% *E.coli*, 36% *K.pneumoniae*, 16% *Enterobacter cloacae (Among the first group of household members)*	NA	First group of household members[a]	Unknown	392	29 years (range: 0-94)	252 (64%)	Closely related: ESBL-PE partially concordant
				Second group of household members[a]	Unknown	361	36 years (range: 10-76)	289 (80%)	
Cross-over index-case based studies									
Rodriguez-Bano J, et al. 2008 [19]	Spain	100% *E. coli*	NA	Index cases (*Outpatients*)	Identified infection	53[b]	69 years (52-75)	37 (70%)	Closely related: ESBL species Clonally related: ESBL strain
				Household contacts	Unknown	73	43 years (23-63)	41 (56%)	
Valverde A, et al. 2008 [23]	Spain	99% *E.coli*, 1% *K.pneumoniae*	NA	Index cases (*Outpatient*)	Identified infection	40	63.6 years (mean) (range: 2-96)	34 (85%)	Closely related: ESBL species Clonally related: ESBL strain
				Household contacts	Unknown	54	NA	NA	
Adler A. et al. 2014. [18]	France, Italy, Spain, Israel	43% *E.coli*, 27% *K.pneumonia*, 16% *P.mirabilis*, 6% *Citrobacter spp.*, 5% *Enterobacter spp.*, 3% others	NA	Index cases (*Inpatient*)	Known colonization	194	65.9 years (mean) (range: 18-99)	98 (50%)	Closely related: ESBL species Clonally related: ESBL strain
				Household contacts	Unknown	286	52 years (42.7-60.2)	204 (71%)	
Liakopoulos A, et al. 2018 [22]	Netherlands	93.7% *E.coli*, 3.75% *Klebsiella pneumoniae,* 2.5% *Enterobacter cloacae*	NA	First group of household members[c]	Known colonization	66	2.4 years (1.5-3.3)	NA	Closely related: ESBL species sharing the same resistance genes Clonally related: ESBL strain
				Second group of household members[c]	Unknown	66	34 years (31-37)	NA	
Longitudinal Index-Case based cohort studies									

Tande D, et al. 2010 [26]	France	56% *E. coli*, unknown proportion of *S.enterica*	12 months (Period prevalence)	Index cases *Outpatient, post-adoption*)	Known colonization	22	NA	NA	Closely related: ESBL-PE Clonally related: ESBL strain
				Household contacts	Unknown	49	NA	NA	
Hilty M, et al. 2012 [29]	Switzerland	88% *E.coli*, 12% *K. pneumoniae*	12 months (Period prevalence[d])	Index cases (*Inpatient & Outpatient*)	Known colonization or infection	82	49 years (mean)	52 (63%)	Closely related: ESBL-Ec and ESBL-Kp Clonally related: ESBL strain
				Household contacts	Unknown	96	NA	NA	
Löhr I.H., et al. 2013 [27]	Norway	100% *K. pneumoniae*	23 months (Period prevalence)	Index cases (*Inpatient, post-outbreak*)	Known colonization	28	Neonates	26 (51%)	Closely related: CTXM-15 species Clonally related: CTXM-15 strain
				Household contacts	Unknown	60	NA	NA	
Strenger V, et al. 2013 [20]	Austria	44% *K. oxytoca*, 28% *S.marcescens*, 24% *K. pneumoniae*, 4% *E.coli*	12 months (Period prevalence[d])	Index cases (*Inpatient*)	Known colonization	25	Neonates	13 (52%)	Closely related: ESBL-PE Clonally related: ESBL strain
				Household contacts	Unknown	49	NA	NA	
Arcilla MS, et al. 2017 [24]	Netherlands	*Enterobacteriaceae (no detail)*	12 months (Not considered for co-carriage)	Index cases (*Outpatient, returning travellers*)	Known colonization	152	NA[e]	NA[e]	Closely related: ESBL-PE- sharing the same group of resistance gene
				Household contacts	Not colonized by ESBL-PE	168	NA[e]	NA[e]	
Haverkate MR, et al. 2017 [28]	Netherlands	66,7% *E.coli*, 17.9% *K.pneumoniae*, 12.8% *Enterobacter cloacae,* 2.6% *Citrobacter freundii*	18 months Period (closely-) and point (clonally-related ESBL PE) prevalence	Index cases (*Inpatient*)	Suspicion of colonization or infection	74	54 years (mean) (SD: 24)	36 (49%)	Closely related: ESBL-PE Clonally related: ESBL strain
				Household contacts	Unknown	84	43 years (mean) (SD: 23)	45 (54%)	
Stewardson AJ et al. 2018 [4]	Belgium, Poland, Switzerland	100% *E.coli*	36.5 days (Point-prevalence)	Index cases considered for co-carriage[f]	Known colonization	33	30 years (19-55)	21 (64%)	Closely related: ESBL species
				Household contacts considered for co-carriage[f]	Unknown	46	39 years (27.2-49)	26 (56%)	
				Index cases considered for acquisition rates[f]	Known colonization	36	NA[e]	NA[e]	
				Household contacts considered for acquisition rates[f]	Free of ESBL-PE at baseline	55	NA[e]	NA[e]	

CTXM: Specific family of genes coding for Extended-Spectrum Beta-Lactamase

CTXM-15: Specific gene coding for Extended-Spectrum Beta-Lactamase

ESBL: Extended-Spectrum Beta-Lactamase

ESBL-PE: Extended-Spectrum Beta-Lactamase Producing *Enterobacteriaceae*

ESBL Ec: Extended-Spectrum Beta-Lactamase Producing *E.coli*

ESBL Kp: Extended-Spectrum Beta-Lactamase Producing *K.pneumoniae*

Footnotes to the table 1:

[a] Caregivers and not household members were concerned.

[b] 13 index cases lived alone.

[c] Data were extracted based on the crude microbiological data from the study "Van den Bunt et al." Epidemiological information on this sub-population (household members of a known carrier) is missing.

[d] Longitudinal cohort study not considered for acquisition rates because unknown proportion of previously negative household members.

[e] Nested cohort from the main study population, with missing epidemiological information.

[f] Co-carriage: One household member positive at baseline per household. Acquisition rates: One household member positive with negative household members.

Co-carriage proportions or acquisition rates were assessed for closely-related and for clonally-related pathogens in 13/13 and 10/13 studies, respectively. Closely-related pathogens were defined as the sharing of same ESBL-PE species,(42,56,57,60,61,65,67) or ESBL-PE without species identification. (58,59,62–64,66) Pathogen characteristics, as well as main features of the applied microbiologic methods are described in Supplementary Table 1. Supplementary Table 2 summarizes reporting practices of the included studies. Potential confounders and biases were mainly reported for index cases at baseline, especially for previous antibiotic intake (12/13 studies) and previous hospital stays (10/13 studies). However, risk factors were often heterogeneously defined, and poorly reported during follow-up of household members. Considering potential microbiological biases (Supplementary Table 3), only 3 studies used broth enrichment,(60,62,65) and 4 analyzed more than one colony per morphotype.(42,57,59,60)

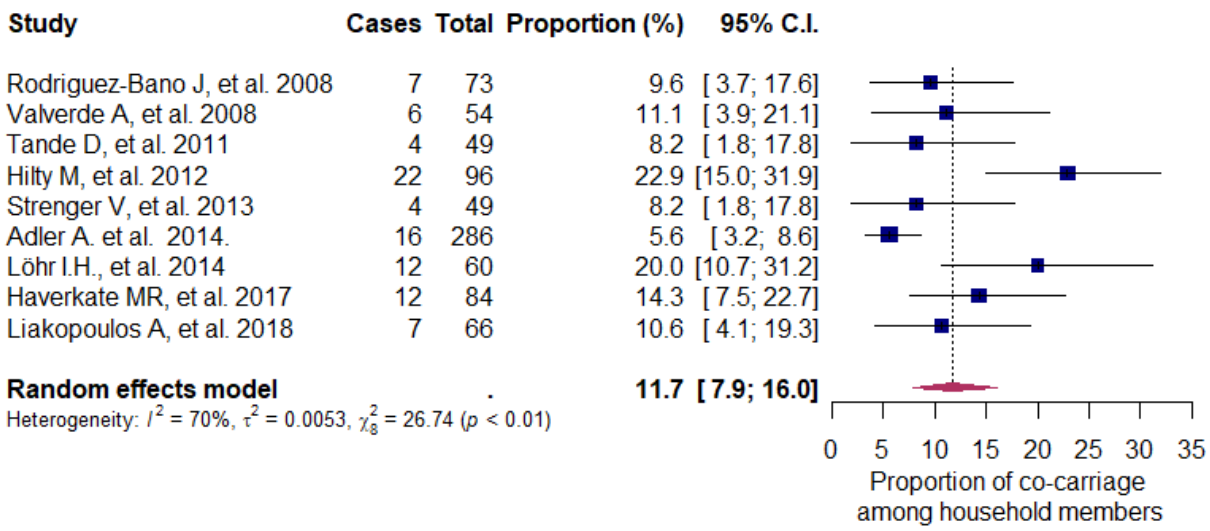
Index-case based studies evaluating co-carriage of ESBL-producing Enterobacteriaceae among household members

Co-carriage proportions of closely-related pathogens were collected as a point-prevalence (either in cross-sectional studies or at baseline of longitudinal studies) in 5 studies and as a period prevalence (with varying follow-up from 12 to 23 months) in 5 longitudinal studies. When considering co-carriage of closely-related pathogens at the species level, point prevalence and period prevalence of ESBL-PE co-carriage among household members of a previously identified index case ranged between 8-27% and 14-34%, respectively. When considering co-carriage of closely-related pathogens at the *Enterobacteriaceae* level, period prevalence of co-carriage among household members of an index case ranged between 18-37%.

In the nine studies assessing co-carriage of clonally-related pathogens, including 817 household members of index cases colonized or infected by ESBL-PE, the proportion of co-carriage with a clonally-related strain ranged between 5.6% and 23% (cf. Supplementary Table 4). The pooled estimate was 12% (95%CI : 8-16%;

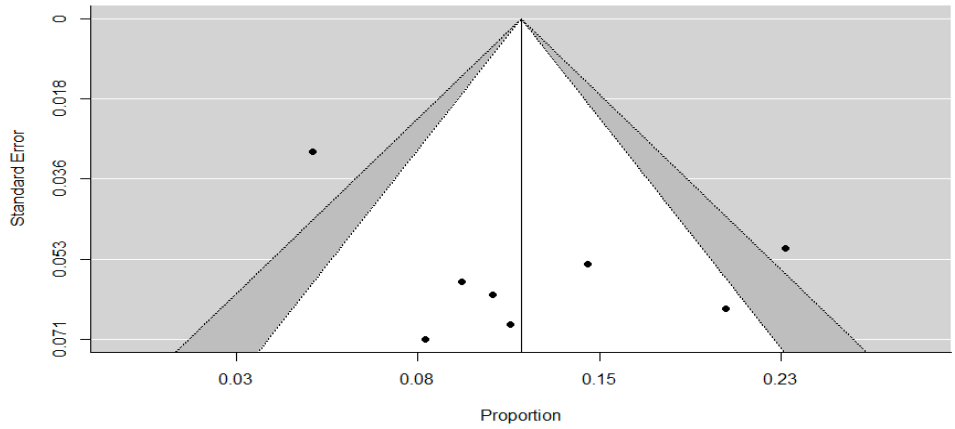
Figure 3). Large heterogeneity was observed among studies (I^2: 70%), with a Q-test for heterogeneity rejecting the hypothesis of homogeneity (P<.001).

Figure 3. Forest plots for prevalence of co-carriage of clonally-related Extended-Spectrum β-lactamases Producing Enterobacteriaceae (ESBL-PE)



Co-carriage proportions of clonally-related *K.pneumoniae* or *E.coli* were evaluated respectively by 2 and 3 studies and ranged between 20-25%(65,67) and 10-20%(57,61,67) revealing important differences after stratification by species. In a subgroup analysis stratifying studies that included <15% vs >15% of index cases colonized by ESBL-producing *Klebsiella spp*., co-carriage proportions were observed to increase for studies including more *Klebsiella spp*. (13%, with 95%CI 7-21% compared to 10% with 95%CI 6-14%, respectively). Inspection of the funnel plot (Figure 4) was not suggestive of any reporting bias for the primary outcome.

Figure 4. Evaluation of potential publication bias, funnel plot for prevalence of co-carriage of clonally-related Extended-Spectrum β-lactamases Producing Enterobacteriaceae (ESBL-PE)



Population-based studies evaluating co-carriage of ESBL-producing Enterobacteriaceae among multiple families

Co-carriage at the population-level was evaluated by 2 studies for closely-related ESBL-PE (prevalence, 15% and 14%), and by a single study for clonally-related ESBL-PE (6%; Supplementary Table 5).

Acquisition rates of ESBL-PE

Follow-up periods in the 5 prospective cohort studies evaluating ESBL-PE acquisition rates ranged from 36 days to 23 months, with a variable frequency between screening time points. Acquisition rates of closely-related ESBL-PE among household members of a previously identified carrier were reported by 2 studies, and ranged between 1.5 and 17.39 events per 1000 person-weeks, by following up 223 initially ESBL-PE free household members. When restricting to clonally-related ESBL-PE reported in 3 studies, the rates ranged between 1.56 and 2.03 events per 1000 person-weeks of follow-up among 180 initially ESBL-PE free household members (Supplementary Table 6). Acquisition rates were slightly higher when expressed as person-weeks at risk, excluding the follow-up time after an acquisition of a related ESBL-PE. In the 3 studies providing detailed data on person-time at risk, the corresponding rates ranged between 1.69 and 19.21

events per 1000 person-weeks at risk versus respectively 1.56 and 17.39 events per 1000 person-weeks of total follow-up.

Discussion

ESBL-PE spread dominates in the community setting, mainly driven by specific subclones of ESBL-producing *E. coli*.(68) Through the sharing of well-recognized risk factors for community ESBL-PE carriage(46–49) and through their daily proximity, household contacts of ESBL-PE carriers are at risk of ESBL-PE acquisition. Household transmission of ESBL-PE has been described, but knowledge of its extent remains scant. This is to our knowledge the first systematic review performed on co-carriage and acquisition of ESBL-PE in private households.

Higher carriage proportions were observed among household members of a colonized or infected index case compared to ESBL-PE carriage prevalence in the general population. For instance, carriage of ESBL-producing *E.coli* and *K.pneumoniae* was 4.5% in the Dutch population,(69) but 18% among such household members.(60,66) In Switzerland, community carriage of ESBL-producing *E.coli* was 5.3%,(70) but up to 34%(67) when considering household members. In France and Spain, community carriage of ESBL-producing *E.coli* was between 2-7%,(71,72) but 14-27% among household members.(57,61,64) When focusing on ESBL-producing *K.pneumoniae*, community carriage was 0.3%(73) in Norway, and 20% in household members of a colonized index case.(65) Thus, families and households may serve as ESBL-PE amplification platforms.

Co-carriage proportions decreased when considering only co-carriage of clonally-related ESBL-PE, with a pooled prevalence of 12%. These findings underline the importance of genotyping methods to elucidate the epidemiology of ESBL-PE in household settings. Moreover, they suggest that multiple sources of ESBL-PE introduction (e.g. food, travel) into households may exist beyond transmission via ESBL-PE index cases that may explain the polyclonal ESBL-PE picture observed in many households.(74)

Confidence intervals of pooled proportions for clonally-related pathogens, as well as the range of prevalence proportions and rates, suggest important variations in co-carriage and acquisition of ESBL-PE between household members. Unfortunately, considering the small sample size and number of studies, there was too much risk of overfitting for subgroup analyses. Several hypotheses might explain, however, this heterogeneity. First, there were substantial differences in study populations, risk factors and microbiological features. For instance, index cases with an ESBL-PE related infection, recruitment after an outbreak with a particularly transmissible strain, as well as antibiotic exposure may all increase the likelihood of ESBL-PE cross-transmission.(42) Additional characteristics of household members, such as healthcare exposure, travel activities and food habits may have influenced ESBL-PE acquisition risks.(42,43,48,49,62) Second, the various study designs lead to different estimates. Co-carriage evaluated during cross-sectional studies and at baseline during longitudinal studies was considered as a point-prevalence proportion. This contrasts with co-carriage evaluated during the whole follow-up of a cohort study, considered as a period-prevalence. Comparability of such proportions might be questionable and may have caused methodological heterogeneity.[37] Third, included studies originated from different regions of the world. However, European households were overrepresented; thus, acquisition rates and co-carriage proportions might differ in other settings, especially in low- and middle-income countries. Clearly, the geographic and socio-economic context influences ESBL-PE colonization pressure, antibiotic exposure, way of living, proximity of household members and ultimately ESBL-PE household acquisition rates.

We identified multiple potential biases in the included studies. Several studies only included two members in household members, introducing selection bias and possibly missing transmission chains. At isolate levels, relatedness analysis was often performed on the basis of a unique isolate per morphotype to determine co-carriage of clonally-related pathogens. Acknowledging co-existence of sensitive and resistant ESBL-PE in our microbiota, some related strains and thus co-carriage has possibly been missed. Another detection bias might have missed resistant

pathogens in the absence of broth-based methods, in case of very low bacterial load. Finally, despite genotyping performed in more than half of included studies, the applied methods were not discriminative enough to assess strain relatedness among isolates, in order to distinguish acquisition from external sources versus cross-transmission. Of upmost importance, none of the included studies performed advanced bacterial or plasmid sequencing using whole genome sequencing to elucidate the exact transmission pathways of ESBL-PE, as already done in hospital-based studies.(76) Only 3 studies examined the spread of plasmids to other species in the gut across family members, in the absence of clonally related pathogens (Supplementary appendix 7). However, the methods were not discriminative enough to ascertain horizontal transfers of mobile genetic elements. Only sporadic sharing of plasmid profiles among household members were observed, but available data were not sufficient to measure the influence of mobile genetic transfer in acquisition rates of antibiotic resistance.

Differences in cross-transmission risk between *Klebsiella* spp and *E. coli* have been described in hospital-based studies.(77) We identified a similar trend in the included household studies. If *Klebsiella* is more transmissible, *E. coli* seems to be a more successful colonizer of humans. This dominance might be explained by the presence of more transmission pathways (food chain, environment), and successful dissemination of particularly virulent sub-clones.(78)

Conclusions

In summary, the observed ESBL-PE co-carriage prevalence and acquisition rates are concerning and may explain in part ESBL-PE spread and persistence among families, along with other determinants. The observed heterogeneity in study designs and populations has contributed to the variability of results and limited the precision of our estimates. The methodological limitations of included studies therefore highlight the need for further research evaluating ESBL-PE co-carriage, acquisition and cross-transmission in households, with standardized selection and follow-up of participants. Furthermore, novel sequencing approaches are required

to ascertain exogenous acquisition of bacteria and plasmids. Such research output could help to provide a broader understanding of ESBL-PE transmission dynamics in a One Health perspective, and ultimately could drive future preventive measures to control ESBL-PE in the community.

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Supplementary Appendix

Search concepts

Search concepts included in study type hedges were identified using the PICO format.

Population:

- Household*, communit*, famil*
- MeSH:
    - Medline: household, community, family caregiver, household and family, outpatient
    - Embase: 'family', 'community car', 'household', outpatient
    - Cochrane: Family, Residence Characteristics, outpatient
- Animal*
- MeSH:
    - Medline: “Animals, Domestic”, Pets, "animal, companion"
    - Embase: “companion animal”, “pet animal”
    - Cochrane: Pets

Exposure:

- ESBL, lactamase
- MeSH:
    - Medline: cephalosporin beta lactamase, beta lactamase, cephalosporin resistance
    - Embase: 'extended spectrum beta lactamase producing enterobacteriaceae', 'extended spectrum beta lactamase', ‘cephalosporin resistance’
    - Cochrane: beta-Lactam Resistance

Outcome:

- Transmiss*, carriage, acquisition, coloniz*, microbiota, molecular AND epidemiolog*
- MeSH:

- Medline: communicable disease transmission, "disease transmission, infectious", microbiota, molecular epidemiology
- Embase: 'microbial colonization', 'acquisition', 'disease transmission', microflora, risk factor, molecular epidemiology
- Cochrane: "Disease Transmission, Infectious", Microbiota, Molecular Epidemiology

Design: all types of observational studies were included

Search strategy

For Medline the following terms were used:

Search	Query
#6	#3 AND #4 AND #5 (filter 1990-2018) (no filter about HUMAN studies because might discard some pertinent studies (SATURN))
#5	(ESBL OR lactamase OR cephalosporin beta lactamase[MeSH Terms] OR beta lactamases[MeSH Terms] OR cephalosporin resistance[MeSH Terms])
#4	(Transmiss* OR carriage OR acquisition OR coloniz* OR microbiota OR molecular epidemiolog* OR communicable disease transmission[MeSH Terms] OR disease transmission, infectious[MeSH Terms] OR microbiota[MeSH Terms] OR molecular epidemiology[MeSH Terms])
#3	#1 OR #2
#2	(Animal* OR animal, domestic[MeSH Terms] OR pets[MeSH Terms] OR animal, companion[MeSH Terms])
#1	(Household* OR communit* OR famil* OR household[MeSH Terms] OR community[MeSH Terms] OR family caregiver[MeSH Terms] OR household and family[MeSH Terms])

For EMBASE the following search strategy was used (after a search for index terms of relevant records):

No. Query Results

#31. #12 AND #18 AND #30 (filter 1990-2018 + embase)

#30. #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29

#29. 'molecular epidemiology'/exp

#28. 'microflora'/exp

#27. 'disease transmission'/exp

#26. 'acquisition'/exp

#25. 'microbial colonization'/exp

#24. 'molecular epidemiolog*':ti,ab,kw

#23. 'microbiota':ti,ab,kw

#22. 'coloniz*':ti,ab,kw

#21. 'acquisition':ti,ab,kw

#20. ‘carriage’:ti,ab,kw

#19. 'transmiss*':ti,ab,kw

#18. #13 OR #14 OR #15 OR #16 OR #17

#17. 'extended spectrum beta lactamase'/exp

#16. 'extended spectrum beta lactamase producing enterobacteriaceae'/exp

#15. ‘cephalosporin resistance’/exp

#14. 'lactamase':ti,ab,kw

#13. 'esbl':ti,ab,kw

#12. #7 OR #11

#11. #8 OR #9 OR #10

#10. ‘companion animal’/exp

#9. ‘pet animal’/exp

#8. ‘animal*’:ti,ab,kw

#7. #1 OR #2 OR #3 OR #4 OR #5 OR #6

#6. 'family'/exp

#5. 'famil*':ti,ab,kw

#4. 'community care'/exp

#3. 'communit*':ti,ab,kw

#2. 'household'/exp

#1. 'household*':ti,ab,kw

For the Cochrane database, the following MeSH terms were used :

ID	Search
#1	"household*":ti,ab,kw
#2	"communit*":ti,ab,kw
#3	"famil*":ti,ab,kw
#4	MeSH descriptor: [Family] explode all trees
#5	MeSH descriptor: [Residence Characteristics] explode all trees
#6	#1 or #2 or #3 or #4 or #5
#7	"animal*":ti,ab,kw
#8	MeSH descriptor: [Pets] explode all trees
#9	#7 or #8
#10	#6 or #9
#11	"ESBL":ti,ab,kw
#12	"lactamase":ti,ab,kw
#13	MeSH descriptor: [Cephalosporin resistance] explode all trees
#14	MeSH descriptor: [beta-Lactam Resistance] explode all trees
#15	#11 or #12 or #13 or #14
#16	"transmiss*":ti,ab,kw
#17	"acquisition":ti,ab,kw
#18	"carriage":ti,ab,kw
#19	"coloniz":ti,ab,kw

#20	"microbiota":ti,ab,kw
#21	"molecular and epidemiolog*":ti,ab,kw
#22	MeSH descriptor: [Disease Transmission, Infectious] explode all trees
#23	MeSH descriptor: [Microbiota] explode all trees
#24	MeSH descriptor: [Molecular Epidemiology] explode all trees
#25	#16 or #17 or #18 or #19 or #20 or #21 or #22 or #23 or #24
#26	#10 and #15 and #25 (1990 - 2018)

Importation of references:

All data were imported in DistillerSR using RIS format. Txt format for Central and Pubmed have been adapted in a RIS-friendly format by https://eppi.ioe.ac.uk/cms/er4/RISExport/tabid/2934/Default.aspx

Pilot-test of the search strategy:

The search strategy was pilot-tested with a subset of relevant studies:

https://www.ncbi.nlm.nih.gov/pubmed/29331548

Effect of outpatient antibiotics for urinary tract infections on antimicrobial resistance among commensal Enterobacteriaceae: a multinational prospective cohort study.

https://www.ncbi.nlm.nih.gov/pubmed/27596534

Quantifying within-household transmission of extended-spectrum β-lactamase producing bacteria.

https://www.ncbi.nlm.nih.gov/pubmed/18641033

Faecal carriage of extended-spectrum beta-lactamase-producing Escherichia coli: prevalence, risk factors and molecular epidemiology.

https://www.ncbi.nlm.nih.gov/pubmed/20233775

Intrafamilial transmission of extended-spectrum-beta-lactamase-producing Escherichia coli and Salmonella enterica Babelsberg among the families of internationally adopted children.

https://www.ncbi.nlm.nih.gov/pubmed/22718774

Transmission dynamics of extended-spectrum β-lactamase-producing Enterobacteriaceae in the tertiary care hospital and the household setting.

https://academic.oup.com/jac/article/72/2/589/2374137

ESBL/AmpC-producing Enterobacteriaceae in households with children of preschool age: prevalence, risk factors and co-carriage

https://www.ncbi.nlm.nih.gov/pubmed/18562591

High rate of intestinal colonization with extended-spectrum-beta-lactamase-producing organisms in household contacts of infected community patients.

https://www.ncbi.nlm.nih.gov/pubmed/20144898

Fecal carriage of CTXM type extended-spectrum beta-lactamase-producing organisms by children and their household contacts.

https://academic.oup.com/jac/article/68/5/1043/682782

		Relevant studies
Search strategy (Pubmed)	Studies retrieved	9
	Studies not retrieved	0
		9

Sensitivity of the search strategy: 100%

533 *Figures & tables*

534

535 *Supplementary table 1. Microbiological methods*

Bibliography	Sample	Broth	Species discrimination	Target pathogen or organism for the index case	Method of resistance determination	Resistance profile included	Technique used to assess relatedness
Rodriguez-Bano J et al. 2008 (57)	rectal swab	no	genotypic	100% *E. coli*	genotypic	ESBL	PFGE,rep-PCR
Valverde A. et al. 2008 (61)	stool culture	no	genotypic	99% *E.coli*, 1% *K.pneumoniae* [A]	genotypic	ESBL	PFGE, multiplex-PCR
Lo W.U. et al. 2010 (59)	stool culture	no	genotypic	Among all participants: 81% *E.coli*, 19% *K. pneumoniae*	genotypic	CTXM	PFGE
Tande D. et al. 2010 (64)	stool culture	no	genotypic	56% *E. coli*, unknown proportion of *S.enterica*	genotypic	ESBL	PFGE
Hilty M. et al. 2012 (67)	stool culture	no	genotypic	88% *E.coli,* 12% *K. pneumoniae*	genotypic	ESBL	PFGE, MLST, rep-PCR
Löhr I.H. et al. 2013 (65)	rectal swab, stool culture	yes	genotypic	100% *K. pneumoniae*	genotypic	CTXM-15	PFGE
Strenger V. et al. 2013 (58)	stool culture	no	genotypic	44% *K. oxytoca*, 28% *S.marcescens*, 24% *K. pneumoniae*, 4% *E.coli*	phenotypic	ESBL	rep-PCR
Adler A. et al. 2014 (56)	rectal swab	no	genotypic	43% *E.coli*, 27% *K.pneumoniae*, 16% *P.mirabilis,* 6% *Citrobacter spp.*, 5% *Enterobacter spp.*, 3% others	genotypic	ESBL	PFGE,MLST
Arcilla MS et al. 2017 (62)	stool culture	yes	phenotypic	*Enterobacteriaceae* (no detail)	genotypic	ESBL	N/A
Haverkate MR, et al. 2017 (66)	stool culture	no	genotypic	66,7% *E.coli*, 17.9% *K.pneumoniae*, 12.8% *Enterobacter cloacae,* 2.6% *Citrobacter freundii*	genotypic	ESBL	rep-PCR
Kurz M.S. et al. 2017 (63)	rectal swab	no	phenotypic	48% *E.coli*, 36% *K.pneumoniae*, 16% *Enterobacter cloacae* [B]	phenotypic	ESBL	Partial concordance
Liakopoulos A. et al. 2018 (60)	stool culture	yes	genotypic	*93.7% E.coli, 3.75% Klebsiella pneumoniae, 2.5% Enterobacter cloacae*	genotypic	ESBL / AmpC	PFGE,MLST, resistance gene, replicon type and subtype
Stewardson AJ et al. 2018 (42)	Stool culture	no	Phenotypic	100% *E.coli*	phenotypic	ESBL	N/A

536

537

AmpC: AmpC Beta-Lactamase

CTXM: Specific family of genes coding for Extended-Spectrum Beta-Lactamase

CTXM-15: Specific gene coding for Extended-Spectrum Beta-Lactamase

ESBL: Extended-Spectrum Beta-Lactamase

ESBL-PE: Extended-Spectrum Beta-Lactamase Producing Enterobacteriaceae

MLST: MultiLocus Sequence Typing

PCR: Polymerase Chain Reaction

PFGE: Pulsed-field Gel Electrophoresis

Rep-PCR: Repetitive element palindromic Polymerase Chain Reaction

Footnotes to the Supplementary table 1:

A Population based study, pathogens isolated from all study participants

B Population based study, pathogens isolated from one cohort of the original study (patients recruited at hospital admission)

556 *Supplementary table 2. Reporting practices of potential biases and confounders in the included studies*

Reference	study design	Study population	Sampling criteria	Previous hospital stay		Antibiotic exposure		Travel		Foodborne		Children in Day care centers		Socio-Economic Status	Loss to follow up
				baseline	follow up	baseline	follow up	baseline	follow up	baseline	follow up	baseline	follow up	baseline	
Rodriguez-Bano J et al. 2008 (57)	cross-sectional study	Index case (Outpatient)	ESBL-PE infection	19/53[A] (36%)	na	38/53[B] (72%)	na	-	na	7[C]	na	-	na	-	NA
		Household member	Convenience sample	6/73[A] (8%)	na	8/73[B] (11%)	na	-	na	8.55[C]	na	-	na	-	
Valverde A. et al. 2008 (61)	cross-sectional study	Index case (Outpatient)	ESBL-PE infection	-	na	18/36[B] (50%)	na	-	na	-	na	-	na	-	NA
		Household member	Convenience sample	-	na	-	na	-	na	-	na	-	na	-	
Lo W.U. et al. 2010 (59)	cross-sectional study	Household members (population based study)	Children with acute respiratory or non-febrile illness	13/53[D] (24.5%)	na	24/53[E] (45%)	na	-	na	-	na	-	na	-	NA
			Whole family	7/172[D] (4.1%)	na	40/172[E] (23%)	na	-	na	-	na	-	na	-	
Tande D. et al. 2010 (64)	longitudinal cohort	Index case (adopted children)	ESBL-PE carriage	-	-	-	-	-	-	-	-	-	-	-	Not detailed (mean follow time available)
		Family member	Whole family	-	-	-	-	-	-	-	-	-	-	-	
Hilty M. et al.	longitudinal	Index case (Inpatient &	Newly detected ESBL-PE	11/82[F] (13%)	-	69/82[E] (84%)	-	-	-	-	-	-	-	-	Not detailed

2012 (67)	cohort	Outpatient)	carriage or infection												
		Household member	Convenience sample	-	-	-	-	-	-	-	-	-	-	-	
Löhr I.H. et al. 2013 (65)	longitudinal cohort	Index case (Inpatient, after an outbreak)	ESBL-PE carriage	na[G]	-	33[H] (79%)	-	-	-	-	-	-	-	-	Not detailed (median follow time available)
		Household member	Convenience sample	-	-	-	-	-	-	-	-	-	-	-	
Strenger V. et al. 2013 (58)	longitudinal cohort	Index case (Inpatient)	ESBL-PE carriage	na[G]	11[I] (44%)	15/25[J] (60%)	4/25[K] (16%)	-	-	-	-	-	-	-	Detailed
		Household member	Convenience sample	-	-	-	-	-	-	-	-	-	-	-	
Adler A. et al. 2014 (56)	cross-sectional study	Index case (Inpatient)	ESBL-PE carriage	190/194[F] (98%)	na	99/194[L] (51%)	na	-	na	-	na	-	na	-	NA
		Family member	Convenience sample	28/286[D] (9.8%)	na	17/286[L] (6%)	na	-	na	-	na	-	na	-	
Arcilla MS et al. 2017 (62)	longitudinal cohort	Index case (Travellers)[M]	ESBL-PE carriers	-[Q]	-	-	-	-	-	-	-	-	-	-	Not detailed
		Household member	Convenience sample	-	-	25/215[E] (12%)	-	188/215 (87%)	-	-	-	-	-	78/215[N] (36.4%)	
Haverkate MR, et al. 2017 (66)	longitudinal cohort	Index case (Inpatient)	Suspicion of ESBL-PE colonization or infection	43/74[A] (58.1%)	-	53/71[O] (75%)	74.6%[O] (53/71) - 10.5% (4/38)	-	-	-	-	-	-	-	Detailed
		Household member	Whole family	4/83[A] (4.8%)	-	4/79[O] (5%)	5.3%[O](4/75) - 1.5% (1/66)	-	-	-	-	-	-	-	

Kurz M.S. et al. 2017 (63)	cross-sectional study	Household members (population based study)	Recruited at hospital admission	69/392[A] (18%)	na	98/390[E] (25%)	na	-	na	221/365[P] (60.5%)	na	-	na	117/389[N] (30.1%)	NA
			convenience sample	-	na	-	na	-	na	-	na	-	na	-	
Liakopoulos A. et al. 2018 (60)	cross-sectional study	Household members (population based study)[Q]	Children	-	na	77/1000[R] (8%)	na	-	na	58/1999[S] (5.7%)	na	4.6% (95IC: 2.7-6.4)	na	2.2%[T] (95IC : 0.6-3.9)	NA
			Parents	-	na	32/1000[R] (3%)	na	-	na	675/996[U] (67.8%)	na	5.8% (95IC: 3.9-7.8)	na	4.7%[T] (95IC : 2.4-7.1)	
Stewardson AJ et al. 2018 (42)	longitudinal cohort	Household members (population based study)[Q]	With an antibiotic exposure	33/300[D] 11%	-	119/300[V] (40%)	-	30/300[W] (10%)	-	4/300[X] (1%)	-	38/300[Y] (13%)	-	7/300[Z] (2%)	Detailed
			Without antibiotic exposure	56/416[D] (13%)	-	97/416[V] (23%)	-	56/416[W] (13%)	-	10/416[X] (2%)	-	38/300[Y] (13%)	-	7/300[Z] (2%)	

ESBL-PE : Extended-Spectrum Beta-Lactamase Producing *Enterobacteriaceae*

Footnotes to the Supplementary table 2:

A Healthcare facility in the last 3 months

B Antibiotic exposure in the last 2 months

C Av. days of chicken consumption in the previous month

D Healthcare facility in the last year

E Antibiotic exposure in the last 3 months

F Referral from another healthcare facility

G Neonatal Intensive Care Units admission

H Antibiotic exposure during hospital stay

I Re-hospitalization during follow-up

J Cefuroxime/ampicillin exposure during hospital stay

K Antibiotic exposure (without detail)

L Antibiotic exposure in the last previous month

M Data available from the main study population, but not for this nested cohort

N No education

O ESBL-selecting antibiotic exposure in the last 3 months (non-including carbapenems)

P Eating meat at least once per month

Q Data not available for the cohort derived in our review, but available for the original cohorts of studies

R Antibiotic exposure in the last 6 previous months

S Vegetarians in the households

T Low SES score

U Chicken consumption more than 4 times per month

V Antibiotic exposure in the last year

W High risk travel reported in the last year

X Number of vegetarians

Y Children <5 years that attend day-care

Z Households with only primary education

558

Supplementary table 3. Potential microbiological biases of the included studies

Bibliography	study design	Potential selection bias	Potential detection bias	
		Colonies analyzed per morphotype	Broth use	sampling
Rodriguez-Bano J et al. 2008 (57)	cross-sectional study	>3 colonies and each distinct morphotype	no	not defined
Valverde A. et al. 2008 (61)	cross-sectional study	1 colony	no	not defined
Lo W.U. et al. 2010 (59)	cross-sectional study	<5 colonies	no	not defined
Tande D. et al. 2010 (64)	longitudinal cohort	1 colony	no	not defined
Hilty M. et al. 2012 (67)	longitudinal cohort	not defined	no	not defined
Löhr I.H. et al. 2013 (65)	longitudinal cohort	1 colony	yes	self-collected
Strenger V. et al. 2013 (58)	longitudinal cohort	not defined	no	not defined
Adler A. et al. 2014 (56)	cross-sectional study	1 colony	no	not defined
Arcilla MS et al. 2017 (62)	longitudinal cohort	1 colony	yes	self-collected
Haverkate MR, et al. 2017 (66)	longitudinal cohort	1 colony	no	not defined
Kurz M.S. et al. 2017 (63)	cross-sectional study	not defined	no	not defined
Liakopoulos A. et al. 2018 (60)	cross-sectional study	<5 colonies	yes	self-collected
Stewardson AJ et al. 2018 (42)	longitudinal cohort	10 colonies	no	self-collected

564 *Supplementary table 4. Index-case based studies evaluating co-carriage of closely-related and clonally-related*
565 *ESBL-producing Enterobacteriaceae among household members*

Reference	Study period	Study design	Country	Broth use	Target pathogen for the index cases	Resistance included	Type of prevalence	Discrimination	Proportion	Co-carriage of
Rodriguez-Bano J et al. 2008 (57)	2005-2006	Cross-sectional study	Spain	no	100% *E. coli*	ESBL	Point prevalence	Closely-related	27.4% (20/73)	ESBL species
								Clonally related	9.6% (7/73)	ESBL strain
Valverde A. et al. 2008 (61)	2004-2005	Cross-sectional study	Spain	no	99% *E.coli*, 1% *K.pneumoniae*	ESBL	Point prevalence	Closely-related	16.7% (9/54)	ESBL species
								Clonally related	11.1% (6/54)	ESBL strain
Tande D. et al. 2010 (64)	2002-2005	Prospective cohort study	France	no	56% *E. coli*, unknown proportion of *S.enterica*	ESBL	Period prevalence (12 months)	Closely-related	14.3% (7/49)	ESBL-PE
								Clonally related	8.16% (4/49)	ESBL strain
Hilty M. et al. 2012 (67)	2008-2009	Prospective cohort study	Switzerland	no	88% *E.coli*, 12% *K. pneumoniae*	ESBL	Period prevalence (12 months)	Closely-related	34.4% (33/96)	ESBL-Ec and ESBL-Kp
								Clonally related	22.9% (22/96)	ESBL strain
Löhr I.H. et al. 2013 (65)	2008-2009	Prospective cohort study	Norway	yes	100% *K. pneumoniae*	CTXM-15	Period prevalence (23 months)	Closely-related	20.0% (12/60)	CTXM-15 species
								Clonally related	20% (12/60)	CTXM-15 strain
Strenger V. et al. 2013 (58)	2007-2008	Prospective cohort study	Austria	no	44% *K. oxytoca*, 28% *S.marcescens*, 24% *K. pneumoniae*, 4% *E.coli*	ESBL	Period prevalence (12 months)	Closely-related	18.4% (9/49)	ESBL-PE
								Clonally related	8.2% (4/49)	ESBL strain
				no		ESBL	Point prevalence	Closely-related	8.0% (23/286)	ESBL species

Adler A. et al. 2014 (56)	2007-2008	Nested cross-sectional study in a prospective cohort study	France, Italy, Spain, Israel		43% *E.coli*, 27% *K.pneumonia*, 16% *P.mirabilis*, 6% *Citrobacter spp.*, 5% *Enterobacter spp.*, 3% others			Clonally related	5.6% (16/286)	ESBL strain
Haverkate M.R. et al. 2017 (66)	2010-2013	Prospective cohort study	Netherlands	no	66,7% *E.coli*, 17.9% *K.pneumoniae*, 12.8% *Enterobacter cloacae*, 2.6% *Citrobacter freundii*	ESBL	Period prevalence (18 months) Point prevalence (baseline)	Closely-related	36.9% (31/84)	ESBL-PE
								Clonally related	14.3% (12/84)	ESBL strain
Liakopoulos A. et al. 2018 (60)	2013-2015	Cross-sectional study	Netherlands	yes	93.7% *E.coli*, 3.75% *Klebsiella pneumoniae,* 2.5% *Enterobacter cloacae*	ESBL / AmpC	Point prevalence	Closely-related	18.2% (12/66)	ESBL species sharing the same resistance genes
								Clonally related	10,6% (7/66)	ESBL strain
Stewardson AJ et al. 2018 (42)	2011-2013	Prospective cohort study	Belgium, Poland, Switzerland	no	100% *E.coli*	ESBL	Point prevalence (baseline)	Closely-related	10.9% (5/46)	ESBL species

CTXM-15: Specific gene coding for Extended-Spectrum Beta-Lactamase

ESBL: Extended-Spectrum Beta-Lactamase

ESBL Ec: Extended-Spectrum Beta-Lactamase Producing E.coli

ESBL Kp: Extended-Spectrum Beta-Lactamase Producing K.pneumoniae

ESBL-PE: Extended-Spectrum Beta-Lactamase Producing Enterobacteriaceae

Supplementary table 5. Population-based studies evaluating co-carriage level of closely-related and clonally-related ESBL-producing Enterobacteriaceae among multiple families

Author	Study date	Design	Country	Prevalence type	Broth	Pathogen included	Resistance included	Strain relatedness	Proportion	co-carriage of
Lo W.U. et al. 2010 (59)	2007-2008	Cross-sectional study	China	Point prevalence	no	Among all participants: 81% *E.coli*, 19% *K. pneumoniae*	CTXM	Both phenotypic (speciation) and genotypic (susceptibility testing)	13.6% (83/225)	CTXM-PE
								Clonally related	5.8% (13/225)	CTXM strain
Kurz M.S. et al. 2017 (63)	2014	nested cross-sectional study in a prospective cohort study	Rwanda	Point prevalence	no	Index case: 48% *E.coli*, 36% *K.pneumoniae*, 16% *Enterobacter cloacae*	ESBL	closely-related	15.4% (116/753)	ESBL-PE partially concordant

CTXM: Specific family of genes coding for Extended-Spectrum Beta-Lactamase

ESBL: Extended-Spectrum Beta-Lactamase

ESBL-PE: Extended-Spectrum Beta-Lactamase Producing Enterobacteriaceae

584 *Supplementary table 6. Index-case based studies evaluating acquisition rates of closely-related and clonally-*
585 *related ESBL-producing Enterobacteriaceae among household members*

Author	Study design	Country	Follow up frequency	Follow up time	Broth	Pathogen included	Resistance included	Strain relatedness	Acquisition rate (among person-days)	Acquisition rate (among person-days at risk)	Acquisition of :	Acquisition event	Household members followed
Tande D, et al. 2010 (64)	Prospective cohort study	France	1M	12 M (median follow up time)	no	*E. coli*, *Salmonella enterica Babelsberg* (56%, unknown proportion of *S.enterica*	ESBL	clonally related	1.56 acquisitions per 1000 person-weeks	1.69 acquisitions per 1000 person-weeks at risk	ESBL strain	4	49
Löhr I.H., et al. 2013 (65)	Prospective cohort study	Norway	1M,3M	23 M (median follow up time for infants and household contacts)	yes	*K.pneumoniae*	CTXM-15	clonally related	2.03 acquisitions per 1000 person-weeks	NA	ESBL strain	12	60
Arcilla MS et al. 2017 (62)	Prospective cohort study	Netherlands	1-2W, 1M, 3M, 6M, 12M	12	yes	Index case: *Enterobacteriacea e* (no detail)	ESBL	closely-related	1.50 acquisitions per 1000 person-weeks	NA	ESBL-PE-sharing the same group of resistance gene	13	168
Haverkate MR, et al. 2017 (66)	Prospective cohort study	Netherlands	3M, 6M, 12M, 18M	18M	no	Gram-negative bacteria (Index case: 67% *E.coli*, 18% *Klebsiella pneumoniae*, 13% *Enterobacter cloacae*)	ESBL	clonally related	2.01 acquisitions per 1000 person-weeks	2.90 acquisitions per 1000 person-weeks at risk	ESBL strain	11	71

Stewardson AJ et al. 2018 (42)	Prospective cohort study	Belgium, Poland, Switzerland	Day 8, day 36	36.5 (days)	no	100% *E. coli*	ESBL	closely-related	17.39 acquisitions per 1000 person - weeks	19.21 acquisitions per 1000 person-weeks at risk	ESBL species	5	55

586

587 *CTXM-15: Specific gene coding for Extended-Spectrum Beta-Lactamase*

588 *ESBL: Extended-Spectrum Beta-Lactamase*

589 *ESBL-PE: Extended-Spectrum Beta-Lactamase Producing Enterobacteriaceae*

R code

```
library(metafor)
library(meta)
library(readxl)
library(ggpubr)
library(ggplot2)
library(boot)
#GENOTYPIC DISCRIMINATION
dat <- read_excel("O:/UPCI/Romain_UPCI/Revue systematique
household transmission/R_outcome 13.02_RM.xlsx", sheet =
"cocarriage_geno2")
#individual estimates with transformation (double-arcsin
transformation)
#WHY double-arcsin ? => low proportions, small sample size
ies.da=escalc(xi= case, ni= total, data=dat, measure="PFT", add=0)
#pooled estimates using random effects, with estimation of between-
study variance estimator using restricted maximum-likelihood estimator
pes.da=rma(yi, vi, data=ies.da, method="REML")
#conversion to original data
pes=predict(pes.da, transf=transf.ipft.hm, targ=list(ni=dat$total))
print(pes)
#taux-squared, I-squared, and their 95IC, Q-statistic
print(pes.da, digits=4)
confint(pes.da, digits=8)
#forest plot
pes.summary=metaprop(case, total, bibli, data=dat, sm="PFT",
method.tau="REML", method.ci="NAsm")
precision=sqrt(ies.da$vi)
forest(pes.summary,
       xlim=c(0,35),
       pscale=100,
       rightcols = FALSE,
```

```
        leftcols = c("studlab", "event", "n", "effect", "ci"),
        leftlabs = c("Study", "Cases", "Total", "Proportion (%)", "95%
C.I."),
        xlab = "Proportion of co-carriage \namong household members",
smlab = "",
        weight.study="random", squaresize=0.5, col.square="navy",
        col.square.lines = "navy",
        col.diamond = "maroon",
        col.diamond.lines = "maroon",
        pooled.totals = FALSE,
        comb.fixed=FALSE,
        fs.hetstat = 10,
        print.tau2=TRUE,
        print.Q=TRUE,
        print.pval.Q=TRUE,
        print.I2=TRUE,
        digits=1,
        sortvar = pubdate)
#Funnel plot avec 95 et 99IC
funnel(pes.da, atransf=transf.ipft.hm, targ=list(ni=dat$total),
        level=c(95, 99), shade=c("white", "gray"))
```

647 *Title and Abstract screening form*

648

Question Text	Answer Text
Type of the study:	Research article (observational, interventional, experimental)
	Review article, recommandation, guideline
Does it include Third-Generation Cephalosporin Resistant (3GC-R) Enterobacteriaceae ?	Yes
	No
	Unclear
Is it a study of human subjects? (non animal, non in-vitro...)	Yes
	No
Are multiple members (including pets) taken from more than one household or family in community?	Yes
	No
	No but case report of one household
	Unclear
I still want to include this study in the background material	Yes
	No

649

650 *Full-reading screening form*

Question Text	Answer Text
Language barrier (if non-EN indicate the language in comments)	Possible to read
	Impossible to read
Type of the study:	Research article (observational, interventional, experimental)
	Review article, recommendation, guideline
Is it a study of human subjects ? (non animal, non in-vitro...)	Yes
	No
Does it include 3rd-Generation Cephalosporin Resistant (3GC-R) *E. coli* and/or *K. pneumoniae* ?	Yes

	No
	Unclear
Are multiple members (including pets) taken from more than one household or family in community?	Yes
	No
	Unclear
	Yes but case report of a single household
Does it only concern: (choose what apply)	Only animal – human transmission but with other animals than pets
	Only animal - human transmission with domestic animals
	Only non-household settings (pig farms, child care facilities, travel, etc…)
	Only mother-to-child transmission (neonatal ≤ 1 month)
	Community outbreak (foodborne...)
	Nothing of the above
Does it analyze prevalence, acquisition, co-carriage or transmission rate between household members and/or pets-household members of 3GC-R *E. coli* and/or *K. pneumoniae* ?	Yes
	No
	Unclear
Any other comment:	

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Plasmidic transfer

Haverkate et al:

Method: PCR-based replicon typing

Definition: different strains sharing the same plasmid incompatibility group and ESBL gene

Results: Among 84 household members at baseline, one shared with an index case the same plasmid incompatibility group and ESBL gene on an unrelated *Klebsiella*. Impossible to determine plasmid acquisition during the follow up because species are not specified in the article.

Liakopoulos et al:

Method: extraction, PCR-based replicon typing, PCR-based replicon sub-typing, PCR-based typing of frequent insertion sequences (ISCR1, ISEcop1, IS26)

Definition: sharing between two different strains of the same ESBL/AmpC gene on the same genetic location on a plasmid belonging to the same replicon type and subtype.

Results: No plasmidic co-carriage between two different strains observed.

Tandé et al:

Method: extraction, electrophoresis

Definition: different strains sharing the same plasmid profile and ESBL gene

Results: no observed plasmid transfer between two different strains observed.

Part 3) Household acquisition and transmission of extended-spectrum β-lactamase (ESBL) -producing Enterobacteriaceae after hospital discharge of ESBL-positive index patients

A similar version of this chapter was published under the following reference:

Riccio ME, Verschuuren Tess, Conzelmann N, Martak D, Meunier A, Salamanca E, Delgado M, Guther J, Peter S, Paganini J, Martischang R, Sauser J, de Kraker MEA, Cherkaoui A, Fluit Ad C, Cooper BS, Hocquet D, Kluytmans JAWK, Tacconelli E, Rodriguez-Baño J, Harbarth S, MODERN WP2 study group. Household acquisition and transmission of extended-spectrum β-lactamase (ESBL) -producing Enterobacteriaceae after hospital discharge of ESBL-positive index patients. Clin Microbiol Infect. 2021;7: 1198-743X(20)30784-9. DOI: 10.1016/j.cmi.2020.12.024.

Abstract

Objectives:

This study aimed to determine rates and risk factors of ESBL-producing *Enterobacteriaceae* (ESBL-PE) acquisition and transmission within households after hospital discharge of an ESBL-PE-positive index patient.

Methods:

2-year prospective cohort study in 5 European cities. Patients colonised with ESBL-producing *Escherichia coli* (ESBL-Ec) or *Klebsiella pneumoniae* (ESBL-Kp), and their household contacts were followed up during 4 months after hospital discharge of the index case. At each follow-up, participants provided a faecal sample and personal information. ESBL-PE whole genome sequences were compared using pairwise Single Nucleotide Polymorphism (SNP)-based analysis.

Results:

We enrolled 71 index patients carrying ESBL-Ec (n=45), ESBL-Kp (n=20) or both (n=6), and 102 households contacts. The incidence of any ESBL-PE acquisition among household members initially free of ESBL-PE was 1.9/100 participant-weeks at risk. Nineteen clonally related household transmissions occurred (case to contact: 13; contact to case: 6), with an overall rate of 1.18 transmissions/100 participant-weeks at risk. Most of the acquisition and transmission events occurred within the first 2 months after discharge. The rate of *ESBL*-Kp household transmission (1.16/100 weeks) was higher than of ESBL-Ec (0.93/100 weeks), whereas more acquisitions were noted for ESBL-Ec (1.06/100 weeks) compared to *ESBL*-Kp (0.65/100 weeks). Providing assistance for urinary and faecal excretion to the index case by household members increased the risk of ESBL-PE transmission (adjusted prevalence ratio, 4.3; 95%CI 1.3-14.1).

Conclusions:

ESBL-PE cases discharged from the hospital are an important source of ESBL-PE transmission within households. Most acquisition and transmission events occurred during the first 2 months after hospital discharge and were causally related to care activities at home, highlighting the importance of hygiene measures in community settings.

Introduction

While transmission of extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-PE) in the clinical setting has been extensively studied (1), little is known about the risk and pathways of transmission in the community. A recent systematic review evaluating human-to-human ESBL-PE transmission between household contacts highlighted important limitations of previous studies (2): low discriminatory power of previously applied typing methods for identifying ESBL-PE transmission events (3); cross-sectional study design preventing the assessment of transmission dynamics over time; and not systematic assessment of ESBL-PE transmission paths and possible epidemiological determinants. Furthermore, only two studies focused on the likelihood of household transmission of ESBL-PE after hospital discharge of an ESBL-positive patient (4).

Specific aims

The aim of this study was to investigate ESBL-PE acquisition and transmission in household settings in five European cities with varying ESBL-PE baseline prevalence. Specifically, we attempted to determine the incidence and risk factors of ESBL-PE acquisition and transmission within families after hospital discharge of an ESBL-PE carrier.

Methods

Study design

We conducted a prospective multicentre cohort study including ESBL-PE positive patients and their household contacts from five university hospitals (Geneva, Sevilla, Tübingen, Utrecht, Besançon). The recruitment target was 20 households by centre (appendix 1, incl. sample size calculation).

Population

Index cases were defined as intestinal ESBL-PE carriers discharged home into a household shared with at least 1 household contact. Household contacts were identified as any person sharing the same household with the index case at least 3 nights a week.

Inclusion and exclusion criteria

The inclusion criteria for the index cases were: to be ≥18 years old; to have a rectal swab or faecal sample at hospital discharge confirming intestinal colonisation with ESBL-producing Escherichia coli (ESBL-Ec) and/or Klebsiella pneumoniae (ESBL-Kp); and to provide informed consent. Patients were excluded if they were permanently institutionalized or impossible to be followed up. After inclusion, index cases were excluded if they had negative rectal samples during the first 2 visits. Enrolled participants who dropped out before collecting the first stool sample were also excluded.

Data collection

All participants were followed up for four months: at hospital discharge (baseline visit #1), 1 week (visit #2), 2 months (visit #3) and 4 months (visit #4). Questionnaires were filled out by all participants at visit #1, #2, #3, and #4. Collected variables concerned participants' health status, antibiotic intake, household conditions, dietary habits and

lifestyle. All participants collected stool samples or rectal swabs by themselves (or a household contact) with ProcultTM 500 kit (Ability Building Centre, Rochester, MN, USA) and faeces containers or Eswabs (Copan Diagnostics, Brescia, Italy) at visit #1, #2, #3 and #4 (±3 days). Collected information was transferred into a centralized REDCap database. The study was approved by each centre's institutional review board.

Microbiologic methods

Selective culturing, enrichment broth, bacterial identification and antimicrobial susceptibility testing were performed for each stool sample or rectal swab at each centre’s microbiology laboratory, using standardized methods (as described in Appendix 2).

Sequencing analysis

The full genome of ESBL-PE isolates was sequenced with NextSeq sequencer (Illumina). DNA extraction was performed with DNeasy UltraClean Microbial Kit (Qiagen). The sequence type (ST) of each isolate was identified by using 7 housekeeping genes, using MLST version 2.10 (https://github.com/tseemann/mlst). ESBL-encoding genes were identified by Resfinder version 2.1 of the Center for Genomic Epidemiology (5). Neighbor-joining core genome multi-locus sequence typing (cgMLST) trees were constructed with SeqSphere+ (Ridom) using the Enterobase scheme (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6961584/) for E.coli (2513 genes) and sensu lato scheme for K.pneumoniae (2358 genes). After removing genes not present in all strains, trees were built by comparing 1’863 and 2’088 genes, respectively. For strains presenting the same cgMLST alongside a strong epidemiological link, pairwise single nucleotide polymorphisms (SNPs) distances were estimated by using the CFSAN pipeline (6).

Definitions

Genomes of ESBL-PE isolates were considered clonally related and closely related, when having respectively a pairwise distance of ≤ 10, or 11-25 SNP differences (7). Acquisition was defined as newly identified carriage of an ESBL-Ec or ESBL-Kp strain during follow-up, not previously detected in the gut flora of the concerned participant. Transmission was defined as the newly detected intestinal carriage of ESBL-Ec and/or ESBL-Kp of a clonally related isolate previously identified in another household member. Co-carriage was defined as the simultaneous carriage by two or more household members of a clonally related isolate at the same sampling time point.

Data analysis

Overall and species-specific incidence rates of acquisition and transmission were estimated at the genotypic level. Time at risk of ESBL-PE acquisition was estimated as the number of days between baseline and the acquisition of the corresponding pathogen in a participant previously free of it, or the dropout of the participant, or end of follow-up, whichever occurred first. The time at risk of a possible ESBL-PE transmission was estimated as the time between baseline (for index cases) or the date of the first positive sample (for household contacts), and the first detection date of a clonally-related isolate previously identified in another household member. Incidence rates were calculated as the total number of acquisition or transmission events divided by the total number of participant-weeks at risk multiplied by 100.

Risk factors of acquisition and transmission were evaluated by univariable and multivariable mixed-effects Poisson regression models to compute prevalence ratios (8, 9), accounting for the lack of independence between repeated samples and multiple clustering effects. The multilevel structure

of the data was composed by three levels: participant (4 samples per participant), household, and study site. Potential confounders were chosen on the basis of existing evidence, and were only scored if exposure preceded the event, with final model selection performed using stepwise backward model selection based on Akaike's information criterion (10). Analyses were performed using R (version 3.6.3.) and STATA version 15 (StataCorp., USA).

Results

Recruitment and household characteristics

Between November 2017 and April 2019, 71 households were included in the study, with 71 index cases and 102 of 127 eligible household contacts (participation rate, 80%). During the 4-month follow-up, 35 participants from 14 households dropped out (Figure 1). Important characteristics of participating households are shown in Table 1. The mean age of all participants was 53±21 years; 47% were female.

Figure 1. Study flow diagram of study participants, by centre and overall.

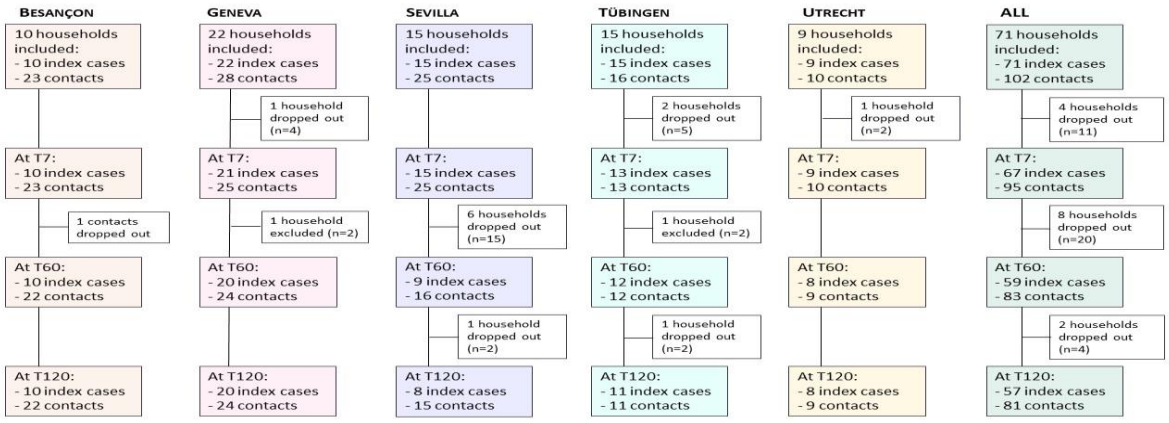


Table 1. Characteristics of households included in the study.

	ESBL-*E. coli*	ESBL-*K. pneumoniae*	*ESBL-E. coli & ESBL-K. pneumoniae*
	N (%)	**N (%)**	**N (%)**
Total N	45	20	6
Study site			
Besançon	7 (15.6)	3 (15.0)	0
Geneva	12 (26.7)	6 (30.0)	4 (66.7)
Sevilla	9 (20.0)	6 (30.0)	0
Tübingen	11 (24.4)	2 (10.0)	2 (33.3)
Utrecht	6 (13.3)	3 (15.0)	0
Number of participating household members			
2	33 (73.3)	14 (70.0)	5 (83.3)
3	7 (15.6)	3 (15.0)	1 (16.7)
>4	5 (11.1)	3 (15.0)	0
Children in the household			
<18 years	9 (20.0)	7 (35.0)	1 (16.7)
<5 years	3 (6.7)	4 (20)	0
Household exposure to at least 2 antibiotics during follow-up			
T60	7 (15.6)	3 (15.0)	0 (0)
T120	7 (15.6)	1 (5.0)	0 (0)
Number of toilets in household			
>2	17 (39.5)	8 (40.0)	3 (60.0)
Bath separated from toilet	16 (36.4)	3 (15.0)	2 (33.3)
Surface of living space, m^2 (median, SD)	122.2 (69.7)	154.2 (82.3)	132 (45.7)
Vegetarians in household	1 (2.3)	1 (5.0)	0

Data are reported in N (%), unless stated otherwise.

Profile of index cases and household contacts

Baseline characteristics of index cases and household contacts are presented in Table 2 and Supplementary Table 1. During hospital stay, 32% (n=23) of index cases had an ESBL-PE infection and 39% (n=28) received antibiotics at hospital discharge.

Table 2. Main characteristics of ESBL-PE positive index cases included in the study.

	ESBL-*E. coli* (n=45)	ESBL-*K. pneumoniae* (n=20)	ESBL-*E. coli* & ESBL-*K. pneumoniae* (n=6)
Demographic			
Age (median, range)	62 (21-89)	64 (28-96)	57.5 (51-83)
Female gender	16 (35.6)	9 (45.0)	2 (33.3)
Highest education			
Primary school	11 (24.4)	7 (35.0)	0
Secondary school	11 (24.4)	8 (40.0)	0
Technical school	11 (24.4)	4 (20.0)	0
University	5 (11.1)	1 (5.0)	5 (83.3)
Other/unknown	7 (15.6)	0	1 (16.6)
Antibiotic exposure in previous 12 months	19 (42.2)	8 (40.0)	1 (16.7)
Travel abroad last 12 months	23 (52.3)	5 (25.0)	4 (66.7)
Dietary habits			
Omnivore	42 (97.7)	19(95)	5(83.3)
Weekly meat consumption	38.5 (86.0)	20 (100)	4 (67)
Vegetarian	1 (2.3)	1 (5.0)	0
Hospital length of stay			
1-7 days	19 (42.2)	3 (15.0)	3 (50.0)

8-14 days	10 (22.2)	6 (30.0)	1(16.7)
15-28 days	8 (17.8)	6 (30.0)	0
>28 days	8 (17.8)	5 (25.0)	2 (33.3)
Comorbidities	40 (88.9)	18 (90.0)	5 (83.3)
Auto-immune disease	0	2 (10.0)	0
Cardio-vascular disease	20 (44.4)	7 (35.0)	2 (33.3)
Chronic dermatologic disease	4 (8.9)	1 (5.0)	1 (16.7)
Chronic renal failure	7 (15.6)	1 (5.0)	0
Chronic obstructive pulmonary disease	3 (6.7)	2 (10)	0
Diabetes	14 (31.1)	3 (15.0)	0
Gastro-intestinal disease	7 (15.6)	3 (15.0)	0
Chronic diarrhoea	1 (2.2)	0	0
Hepatic disease	4 (8.9)	2 (10.0)	0
Inflammatory bowel disease	3 (6.7)	2 (10)	0
Hemiplegia	0	1 (5.0)	0
Immunosuppression	5 (11.1)	4 (20.0)	1 (16.7)
Malignancy	14 (31.1)	9 (45.0)	1 (16.7)
Other	19 (42.2)	10 (50.0)	4 (66.7)
ESBL-PE infection during hospitalisation			
Yes	15 (33.3)	5 (25.0)	3 (50.0)
No	26 (57.8)	13 (65.0)	3 (50.0)
Unknown	4 (8.9)	2 (10.0)	0
Antibiotics at discharge			
Yes	19 (42.2)	8 (40.0)	1 (16.7)
No	26 (57.8)	11 (55.0)	4 (66.7)
Unknown	0	1 (5.0)	1 (16.7)
Incontinence	6 (13.3)	6 (30.0)	0

	Urinary incontinence	3 (6.7)	4 (20.0)	0
	Faecal incontinence	2 (4.4)	2 (10.0)	0
	Both	1 (2.2)	0	0
Indwelling device at discharge		34 (75.6)	12 (60.0)	5 (83.3)
	Intravascular	4 (8.9)	4 (20.0)	1 (16.7)
	Urinary	1 (2.2)	2 (10.0)	0
	Other	7 (15.6)	2 (10.0)	0
Patient autonomy				
	Not completely autonomous	19 (42.2)	11 (55.0)	3 (50.0)
	Needs support by family members	12 (26.7)	8 (40.0)	2 (33.3)
	Help required for urinary or faecal excretion	2 (4.4)	6 (30.0)	0
	Home care by healthcare personnel	12 (26.7)	5 (25.0)	1 (16.7)

Data are reported in N (%), unless stated otherwise.

ESBL-PE carriage and acquisition

At baseline, index cases were carrying ESBL-Ec (n=45, 63%) or ESBL-Kp (n=20, 28%) or both (n=6, 8%). Among household contacts already positive at baseline (n=29, 31%), 79% (23/29) were carrying the same ESBL-PE as their corresponding index case. Twenty-six percent (17/65) of household contacts with complete follow-up acquired ESBL-PE (ESBL-Ec, 11; ESBL-Kp, 6). Most ESBL-PE acquisitions occurred during the first 2 months (1st week: 41%; 2nd-8th week: 29%). One third of index cases (n=27) were ESBL-PE negative at the end of follow-up.

Genetic profiles

Overall, 38 different STs were observed for ESBL-Ec and 29 for ESBL-Kp (Suppl. Figure 1). Among ESBL-Ec strains, ST131 was the most frequent ST (46%). Less frequent STs were ST38 (6.9%), ST1193 (4%), and ST10

(3.6%). STs from ESBL-Kp showed a large heterogeneity (Suppl. Figure 2). Of 44 different ESBL-encoding genes identified, the most frequent was blaCTX-M-15, detected in 142 ESBL-Ec and 79 ESBL-Kp isolates.

Clonally related co-carriage and transmission of related isolates

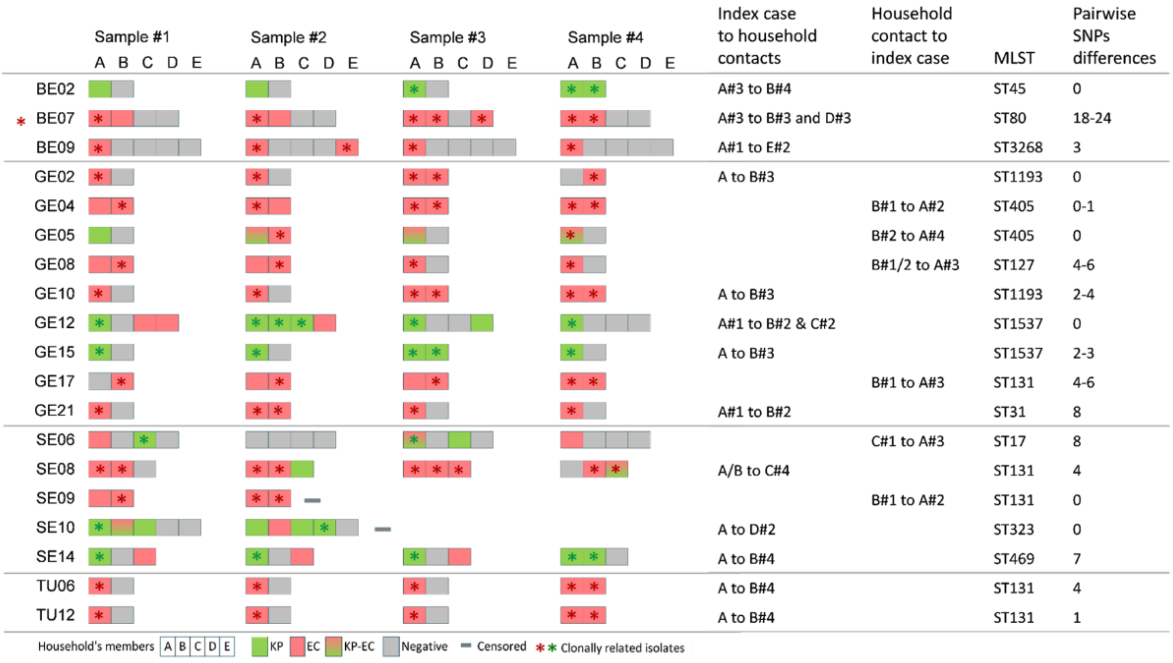
At baseline, 14 out of 29 positive household contacts had isolates clonally related to the index case. The overall prevalence of co-carriage of clonally related isolates was 34% (32/94) over the entire study period.

By combining epidemiological information with WGS data (Figure 2), 19 clonally related transmission events were identified showing two possible directions: from the index case to his/her household contacts (n=13) and vice versa (n=6). Two additional closely related transmission events were identified for household BE07 from Besançon (18 to 24 SNP differences). The isolates belonged to ST80 and the intra-individual genome variability of the ESBL-Ec isolates retrieved from the index case throughout all sampling points ranged from 7 to 11 SNP differences. Most of the transmissions involved ESBL-Ec (14/21), with 9 of them transmitted by the index case (Table 3 and Suppl. Table 2). Fifteen of 21 (71%) transmission events occurred during the first 2 months of follow-up. The phylogenetic trees of retrieved ESBL-Ec and ESBL-Kp strains are shown in Suppl. Figures 3 and 4.

Figure 2. Transmission events of clonally related and closely related isolates of ESBL-producing E. coli and K. pneumoniae, with direction of the transmission pathways. The Figure gives the ST of the transmitted strains and pairwise SNP differences between the concerned isolates.

Each line of the table contains the information for a single household. Each square box represents a sample from a participant at a given sampling time point (i.e. #1, #2, #3, #4). Red and green colours correspond to samples positive with ESBL-producing E. coli and K.

*pneumoniae, respectively. Grey colour corresponds to samples negative for ESBL-PE. Transmission events were identified in two directions: from index case (A) to household members (B to E) and from household contacts to index case. Red boxes (with *) represent clonally related ESBL-E. coli strains and green boxes (with *) represent clonally related ESBL-K. pneumoniae.*



Incidence rates of household acquisition and transmission of ESBL-PE

The overall ESBL-PE acquisition rate was 1.9/100 participant-weeks at risk (Table 3). ESBL-Ec had a higher rate of acquisition than ESBL-Kp. (1.06 vs 0.65/100 participant-weeks at risk; RR 1.65; 95%CI 0.69–3.95). The rate of any clonally related ESBL-PE transmission within households was 1.18 events/100 participant-weeks of follow-up, with the corresponding figure for transmissions only from the index case to household contacts of 0.8/100 weeks (Table 3). Although not statistically significant, a higher overall transmission rate was observed for ESBL-Kp than for ESBL-Ec (1.16 versus 0.93 per 100 participant-weeks at risk; RR 1.25; 95%CI 0.42–3.44) considering all possible transmission paths. A higher rate of ESBL-Kp transmission was also observed from index cases to household contacts (RR 1.87; 95%CI 0.52–6.49).

Table 3. Crude numbers and incidence rates of acquisition and transmission events, based on cgMLST with pairwise SNP differences. ESBL-Ec: ESBL-producing Escherichia coli; ESBL-Kp: ESBL-producing Klebsiella pneumoniae

	Acquisitions from any source			**Transmissions in any direction**			**Transmissions from index case to household contacts**		
	ESBL-Ec	**ESBL-Kp**	**ESBL-PE**	**ESBL-Ec**	**ESBL-Kp**	**ESBL-PE**	**ESBL-Ec**	**ESBL-Kp**	**ESBL-PE**
Crude number	13	12	17	12	7	19	7	6	13
Incidence rate (per 100 participant weeks at risk)	1.06	0.65	1.90	0.93	1.16	1.18	0.53	1.00	0.80

Risk factors for ESBL-PE acquisition and transmission

By univariable, mixed-effects Poisson regression, multiple explanatory factors were significantly associated with the risk of acquiring ESBL-PE among previously ESBL-PE-free household contacts (Suppl. Table 3): (1) index case determinants: hemiplegia, faecal incontinence, previous abdominal infection, proton pump inhibitor therapy, ≥ 3 antibiotic courses after discharge, additional hospitalizations, and assistance provided by household members, in particular for urinary and faecal excretion; (2) household member determinants: age > 50 years; travel abroad; assistance provided by healthcare personnel; help requested for various activities; regular contact with domestic animals; meat and seafood exposure; as well as the number of antibiotic courses. By multivariable

analysis in a parsimonious model, assistance provided by family members to the index case (adjusted prevalence ratio [aPR], 2.9; 95%CI 1.1-8.0) showed the strongest association with ESBL-PE household acquisition, whereas frequency of meat consumption (aPR, 1.4; 95%CI 0.4-5.3) and antibiotic exposure (aPR, 1.4; 95%CI 0.4-4.2) showed only weak evidence of a positive association.

Fourteen variables were found to be significantly associated with the risk of ESBL-PE transmission from the index case to household members in the univariable analysis (Suppl. Table 4): (1) index case determinants: higher education (protective), full autonomy (protective), malignancy, faecal incontinence, previous abdominal infection, urinary catheter, proton pump inhibitor therapy, ≥ 3 antibiotic courses, ≥ 2 hospitalizations, and assistance provided by family members, in particular for urinary and faecal excretion; (2) household member determinants: spouse of index case, antibiotic intake and active helper of index case. In the final multilevel Poisson regression model, assistance provided by household members for urinary and faecal excretion was strongly associated with increased risk of ESBL-PE transmission (aPR, 4.3; 95%CI 1.3-14.1), while household antibiotic exposure showed weaker evidence of a positive association (aPR, 2.1; 95%CI 0.7-7.0).

Discussion

The principal findings of this international cohort study were: (1) clonally related ESBL-PE household transmission after hospital discharge of an ESBL-PE carrier occurred in 19 of 94 participants; (2) most acquisition and transmission events were observed during the first 2 months; (3) other household members were potential sources of cross-transmission, but to a lesser degree; (4) the ESBL-PE acquisition rate was higher than the transmission rate; thus, exogenous acquisition events occurred even without intra-household transmission; (5) the rate of household transmission was higher for ESBL-Kp than for ESBL-Ec; and (6)

assistance provided by family members for urinary and faecal excretion of the index case was the most important risk factor for ESBL-PE transmission.

A recent meta-analysis examining clonally related ESBL-PE among household members documented co-carriage proportions of 12% [95%CI, 8 – 16%], and acquisition rates ranging from 0.16 to 0.20 events/100 participant-weeks of follow-up (2). In contrast, our study observed higher co-carriage proportions (34%) and 10-fold higher acquisition rates (1.9 events per 100 weeks at risk). The higher proportion of co-carriage in the present study might have been influenced by sampling and detection methods, since the use of enrichment broths and selection of multiple colonies per sample might have improved the yield. Furthermore, it may reflect a higher risk of ESBL-PE transmission within enrolled households prior to study participation. The differences in acquisition rates depend on the length of follow-up: longer follow-up periods result in smaller rates. Indeed, 12-month follow-up studies found lower acquisition rates in contrast to shorter follow-up studies, which reported acquisition rates of up to 1.74 closely-related ESBL-PE/100 person-weeks (2, 8, 11). Furthermore, the higher proportion of infected, dependent and antibiotic-treated index cases in our study might have increased early transmission risk for household members compared to previous studies.

The incidence of ESBL-Ec acquisition was higher than the rate for ESBL-Kp. In contrast, household transmission rates were higher for ESBL-Kp compared to ESBL-Ec. This apparent contradiction is explained by the acquisition of ESBL-Ec from a wide range of sources (e.g. food, animals, travel) (12, 13), while transmission, as defined here, only involved human-to-human transfer. Similar observations have also been described for healthcare settings, suggesting that biological differences between bacterial species could explain higher ESBL-Kp transmission rates (14,

15). An alternative explanation might be the slightly higher intra-species diversity of ESBL-Ec within households (mean number of different STs observed per family: 1.6 in ESBL-Ec versus 1.3 in ESBL-Kp). Furthermore, the frequency and intensity of human interactions may facilitate transmission of ESBL-KP, especially among elderly patients (16). Indeed, in our study, index patients carrying ESBL-Kp were sicker and more dependent on external care, leading to increased proximity and risk of transmission.

As Enterobacteriaceae are colonisers of the intestinal tract, the faecal-oral route plays an important role in the transmission chain. As in healthcare settings, where hand hygiene has been shown to be a key factor to reduce pathogen transmission (17), general hygiene measures rather than decreased intake or inappropriate handling of contaminated food may become an important preventive measure to reduce ESBL-PE transmission within households, especially if family members provide assistance to a sick relative (18).

Hitherto, no previous study with these design characteristics and high-resolution typing methods has been conducted in high-income settings to ascertain putative transmission events within entire families, although ESBL-PE acquisition and transmission in the community or low-income settings has previously been investigated (11, 12, 19-24). Therefore, the present study provides a solid methodological foundation for future studies and prioritization of infection control interventions in the community setting.

Several limitations of this study merit consideration. First, not all members living in the same household participated in the study, omitting possible transmission events. Fortunately, the participation rate was high enough (80%) to draw meaningful conclusions. Second, by choosing not more than 4 colonies from a faecal sample, clonally distinct strains might

have been missed, introducing a possible selection bias and underestimating the true transmission rate. As observed in few participants (16%), each host may carry several ESBL-E.coli strains simultaneously. However, we hypothesise that isolates not retrieved might present a low inoculum with lower transmission risk compared to dominating ESBL-E.coli strains. Third, we did not yet conduct plasmid typing, which is part of a complementary investigation, providing a more comprehensive picture of ESBL transmission in the community, especially for E. coli. Fourth, the role of intermediate vectors (i.e. animal) or environmental reservoirs (i.e. surfaces, water, etc) in ESBL-PE transmission was not directly examined, but assumed as a part of direct human-to-human transmission. However, fomite-mediated transmission was accounted for in the estimation of exogenous risk factors by collecting relevant epidemiologic information. Fifth, participants' intestinal load of ESBL-PE was not quantified preventing the consideration of the inoculum effect as an independent risk factor. However, the bacterial load is influenced by several factors that were collected and accounted for in the analysis (e.g. antibiotic exposure, hospital length of stay).

Conclusions

In summary, ESBL-PE carriers discharged from the hospital were an important source of ESBL-PE transmission within households. Most acquisition and transmission events occurred during the first two months after hospital discharge. They were associated with care activities at home, highlighting the importance of hygiene measures to prevent community spread.

References

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Supplementary Appendix

Appendix 1 (sample size calculation):

The sample size was determined for the primary outcome without a pre-specified a priori hypothesis for the risk factor analysis. We assumed an ESBL-PE transmission rate of 10-20% among household members, a cluster size (i.e. number of individuals per household) of 3 and an intraclass correlation coefficient of 0.20 due to the clustering of individuals within families. With a ratio of 1:1 of ESBL-*E. coli* and ESBL-*K.pneumoniae* cases, the planned sample size of 100 index patients (with at least 1 household member) was considered sufficient for the purpose of this observational cohort study.

Appendix 2 (microbiologic methods):

Faecal samples and swabs were streaked directly on ChromID ESBL agar (bioMérieux, Marcy l'Etoile, France) plus additionally in MacConkey broth supplemented with vancomycin 64 µg/mL and 32 µg/mL cefuroxime, incubated for 24 h at 35°C. Centres using rectal swab had verified visually the presence of faecal material in sampling tubes (i.e. white swab tips having brownish stains). As stated by several expert sources, correctly performed rectal swabs remain « an acceptable and practical proxy for the collection of faecal specimens for stool microbiota analysis » (Basis CM et al. Comparison of stool versus rectal swab samples and storage conditions on bacterial community profiles. BMC Microbiology. 2017. DOI: 10.1186/s12866-017-0983-9). Ten µl of the broth was then streaked on ChromID ESBL agar and further incubated for 48 h at 35°C. Each colony morphology was identified using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF). ESBL production was confirmed by double disk synergy tests (DDST20 and DDST30) and by the determination of the β-lactamase inhibition profile (ESBL + AmpC Screen ID Kit, Rosco Diagnostica, Taastrup, Denmark). Based on distinct

colony morphology, each centre stored at -80°C 1 to 4 isolates per sample in bead-containing cryotubes (Microbank, PRO-LAB Diagnostics, ON, Canada) until further analysis.

Supplementary table 1. Main characteristics of participating household contacts.

		ESBL-*E. coli*	ESBL-*K. pneumoniae*	ESBL-*E. coli* & *ESBL-K. pneumoniae*
		(n=63)	(n=32)	(n=7)
Demographics				
	Age (median, range)	54 (2-79)	41 (1-92)	55 (26-84)
	Female gender	36 (57.1)	16 (50)	3 (42.9)
Highest educational level				
	Primary school	16 (25.4)	12 (37.5)	0
	Secondary school	11 (17.5)	7 (21.9)	0
	Technical school	15 (23.8)	6 (18.8)	0
	University	9 (14.3)	5 (15.6)	5 (71.4)
	Other/unknown	12 (19.1)	2 (2.3)	2 (28.6)
Healthcare and antibiotic exposures in previous 12 months				
	Hospitalization	1 (1.6)	1 (3.1)	2 (28.6)
	Antibiotics last 12 months	19 (30.2)	5 (15.6)	1 (14.3)
	Antibiotics at enrolment	2 (3.2)	0	0
Travel abroad last 12 months		30 (48.4)	9 (28.1)	5 (71.4)
Dietary habits				
	Omnivore	57 (90.5)	29 (90.6)	6 (85.7)
	Vegetarian	1 (1.6)	2 (6.2)	0
Relation to the index case				
	Spouse	38 (60.3)	17 (53.1)	6 (85.7)
	Daughter/son	20 (31.8)	14 (43.8)	1 (14.3)
	Parent	1 (1.6)	0	0
	Sibling	1 (1.6)	0	0
	Grand-parent	1 (1.6)	0	0
	Parent in law	0	1 (3.1)	0
	No relationship	2 (3.2)	0	0

Data are reported in N (%), unless stated otherwise.

Supplementary Table 2. Clonally (n=19) or closely (n=2) related transmission events confirmed by analysis of cgMLST and SNP differences. For each centre, it shows the number of ESBL-PE transmission events identified for ESBL-E. coli (ESBL-Ec) and ESBL-K. pneumoniae (ESBL-Kp), at first week (#2), two months (#3) and four months (#4) of follow-up.

Genotypically confirmed transmission		Sample #2		Sample #3		Sample #4		Total
		ESBL-Ec	ESBL-Kp	ESBL-Ec	ESBL-Kp	ESBL-Ec	ESBL-Kp	
Besançon	TOTAL	1		2			1	4
	index case to members	1		2			1	4
	members to index case							0
	members to members							0
Geneva	TOTAL	2	2	4	1	1		10
	index case to members	1	2	2	1			6
	members to index case	1		2		1		4
	members to members							0
Sevilla	TOTAL	1	1	0	1	1	1	5
	index case to members		1			1	1	3
	members to index case	1			1			2
	members to members							0
Tübingen	TOTAL	0	0	0	0	2		2
	index case to members					2		2

	members to index case							0
	members to members							0
Utrecht	TOTAL	0	0	0	0	0		0
	index case to members							0
	members to index case							0
	members to members							0
TOTAL		4	3	6	2	4	2	21

Supplementary Table 3. Risk factors of acquisition of ESBL-PE by previously ESBL-free household contacts (n=17), stratified by index patient versus household variables, including characteristics present at baseline and during follow-up, analysed by univariable mixed effects Poisson regression.

		Exposure variable	Prevalence ratio	Std. Err.	z	P>|z|	95% Conf. interval	
Household characteristics		Household surface >100m^2	0.99	0.40	-0.03	0.98	0.45	2.18
		More than 1 toilet per household	1.09	0.71	0.13	0.89	0.30	3.94
		≥3 Household members	0.61	0.34	-0.88	0.38	0.21	1.81
		Presence of children ≤3 years old	0.92	0.26	-0.30	0.77	0.53	1.59
Index case	At baseline	Age of index case > 50	1.17	0.24	0.75	0.45	0.78	1.74
		Gender (male)	0.80	0.33	-0.53	0.60	0.36	1.80
		Nationality (non-Swiss)	2.69	1.40	1.90	0.06	0.97	7.46
		Higher education	0.79	0.18	-1.02	0.31	0.50	1.24
		Absence of comorbidities	0.64	0.87	-0.33	0.74	0.05	9.07
		Chronic renal failure	1.15	0.43	0.38	0.70	0.56	2.38
		Cardio-vascular disease	1.36	0.84	0.50	0.62	0.41	4.59
		Diabetes	1.83	0.79	1.39	0.16	0.78	4.26
		Hemiplegia	5.35	1.14	7.87	<0.001	3.52	8.13
		Chronic dermatologic disease	0.62	0.47	-0.64	0.52	0.14	2.71
		Chronic obstructive pulmonary disease	1.29	1.22	0.27	0.79	0.20	8.24
		Immunosuppression	1.03	0.50	0.05	0.96	0.39	2.67
		Gastrointestinal disease	0.36	0.38	-0.98	0.33	0.05	2.80
		Malignancy	1.45	0.82	0.67	0.50	0.48	4.37

		Inflammatory bowel disease	0.84	1.01	-0.14	0.89	0.08	8.86
		Any incontinence	1.97	0.99	1.36	0.18	0.74	5.28
		Faecal incontinence	3.00	0.71	4.66	<0.001	1.89	4.76
		Urinary incontinence	1.15	0.32	0.51	0.61	0.67	1.98
		No indwelling device at hospital discharge	0.65	0.22	-1.29	0.20	0.34	1.25
		Urinary catheter at hospital discharge	1.79	0.81	1.30	0.20	0.74	4.33
		Intravascular catheter at hospital discharge	0.83	0.46	-0.33	0.74	0.28	2.48
		Complete autonomy	0.92	0.27	-0.30	0.77	0.52	1.62
		Infection with ESBL-producing organisms during the last hospitalization	0.72	0.30	-0.78	0.44	0.32	1.65
		Infection site: urinary tract	0.82	0.17	-0.97	0.33	0.55	1.22
		Infection site: abdominal tract	1.75	0.40	2.45	0.01	1.12	2.73
		Antibiotic therapy at discharge	1.64	0.51	1.59	0.11	0.89	3.01
	During follow-up	1 additional antibiotic course	1.90	0.90	1.35	0.18	0.75	4.83
		2 additional antibiotic courses	1.03	0.80	0.03	0.98	0.22	4.74
		3 additional antibiotic courses	2.43	0.99	2.19	0.03	1.10	5.38
		Proton pump inhibitors	1.90	0.45	2.70	0.01	1.19	3.02
		H2-receptor antagonists	0.71	0.95	-0.26	0.80	0.05	9.95
		Oral corticosteroids or other immunosuppressive drugs	0.76	0.63	-0.33	0.74	0.15	3.92
		1 additional hospitalization	2.03	0.78	1.84	0.07	0.95	4.32
		2 additional hospitalizations	2.43	1.03	2.10	0.04	1.06	5.57
		Urinary incontinence	1.47	0.73	0.78	0.44	0.56	3.90
		Faecal incontinence	2.25	1.03	1.78	0.08	0.92	5.51
		Indwelling device	2.50	1.22	1.87	0.06	0.96	6.52
		Urinary catheter	2.17	0.99	1.70	0.09	0.89	5.33
		Completely autonomous	0.29	0.25	-1.43	0.15	0.05	1.57
		Help provided by healthcare professional	3.02	2.32	1.44	0.15	0.67	13.58

		Help provided by family members	2.91	1.26	2.48	0.01	1.25	6.78
		Help needed for food preparation	1.11	0.65	0.19	0.85	0.36	3.48
		Help needed for feeding	1.29	0.51	0.65	0.51	0.60	2.81
		Help needed for medication intake	1.96	1.14	1.16	0.25	0.63	6.15
		Help needed for urinary and faecal excretion	3.00	1.18	2.79	0.01	1.39	6.50
		Help needed for dressing	0.97	0.73	-0.04	0.97	0.22	4.21
		Help needed for bed position shift	2.11	1.07	1.46	0.14	0.77	5.73
		Shared bath towel with other family members	1.16	0.35	0.51	0.61	0.65	2.10
		Prepared food for the other household members	0.83	0.33	-0.46	0.64	0.38	1.81
		Cleaned hands before and while cooking meat products	0.65	0.60	-0.46	0.64	0.11	4.00
		Stored separated raw and cooked food	0.56	0.37	-0.88	0.38	0.15	2.05
		Cleaned surfaces and materials used to cook between each meat preparation	0.54	0.51	-0.65	0.52	0.09	3.43
		Used different cooking utensils for raw and cooked food	0.48	0.22	-1.58	0.11	0.19	1.19
Household member	**Baseline**	Gender (male)	1.14	0.40	0.37	0.71	0.57	2.27
		Age household member > 50	1.61	0.18	4.16	<0.001	1.29	2.01
		Higher education	0.84	0.13	-1.13	0.26	0.63	1.13
		Spouse of index case	1.35	0.54	0.74	0.46	0.62	2.94
		Son/daughter of index case	0.54	0.18	-1.82	0.07	0.27	1.05
		Vegetarian	1.08	0.29	0.26	0.79	0.63	1.84
		Number of travels outside Switzerland	1.16	0.09	1.98	0.05	1.00	1.34
	Follow-up	Helper of the index case during follow-up	1.74	0.93	1.03	0.30	0.61	4.97
		Help provided by healthcare professional	3.71	0.76	6.37	<0.001	2.48	5.55
		Help provided by family member	1.79	1.30	0.80	0.42	0.43	7.44
		Help needed for food preparation	2.75	1.33	2.08	0.04	1.06	7.11
		Help needed for feeding	3.71	1.42	3.41	<0.001	1.75	7.87

		Help needed for urinary and faecal excretion	2.75	1.76	1.58	0.11	0.78	9.64
		Help needed for dressing	2.17	1.36	1.24	0.21	0.64	7.42
		Help needed for any mobility	3.71	1.42	3.41	<0.001	1.75	7.87
		Regular contact with domestic animals	0.64	0.11	-2.55	<0.001	0.45	0.90
		Regular contact with cat	1.41	0.24	2.01	0.04	1.01	1.97
		Swim in a river or lake	0.92	0.65	-0.12	0.90	0.23	3.66
		Share towel	0.97	0.16	-0.18	0.86	0.71	1.33
		Eat at least once per week: beef	1.50	0.66	0.93	0.35	0.64	3.54
		Eat at least once per week: lamb	3.14	0.44	8.24	<0.001	2.39	4.12
		Eat at least once per week: pork	1.50	0.27	2.28	0.02	1.06	2.14
		Eat at least once per week: poultry	1.41	0.25	1.89	0.06	0.99	2.00
		Eat at least once per week: fish	1.91	0.68	1.81	0.07	0.95	3.85
		Eat at least once per week: other seafood	2.56	0.72	3.33	<0.001	1.47	4.46
		Spent time cooking meat products	1.12	0.81	0.16	0.88	0.27	4.61
		Prepare food for other household members	1.07	0.59	0.13	0.90	0.37	3.15
		Use different cooking utensils for raw and cooked food	1.02	0.51	0.05	0.96	0.39	2.71
		Number of antibiotic courses	2.18	0.38	4.44	<0.001	1.55	3.07

Supplementary Table 4. Risk factors of clonally related ESBL-PE household transmission from index case to household contacts (n=13), analysed by univariate mixed-effects Poisson regression, stratified by index patient versus household variables, including characteristics present at baseline and during follow-up

Potential risk factors of transmission			**Prevalence ratio**	**Std. Err.**	**z**	**P>z**	**[95% Conf. Interval]**	
Household characteristics		Age	0.78	0.26	-0.74	0.46	0.40	1.51
		Household surface <100m^2	1.03	0.51	0.07	0.95	0.40	2.69
		Main bathroom separated from the toilet	0.52	0.29	-1.17	0.24	0.17	1.55
		Number of toilets in the household	1.43	0.37	1.39	0.17	0.86	2.36
		Number of household members	0.85	0.14	-0.94	0.35	0.62	1.19
		Presence of infants ≤ 3 years old	1.35	1.14	0.35	0.73	0.26	7.09
Index case	**At baseline**	Absence of comorbidities	0.43	0.57	-0.63	0.53	0.03	5.72
		Chronic obstructive pulmonary disease	1.85	1.66	0.69	0.49	0.32	10.76
		Cardio-vascular disease	1.03	0.55	0.06	0.95	0.36	2.93
		Chronic dermatologic disease	0.89	0.64	-0.16	0.87	0.22	3.64
		Diabetes	2.79	1.67	1.72	0.09	0.87	8.99
		Malignancy	2.63	1.29	1.97	0.05	1.01	6.90
		Inflammatory bowel disease	1.22	1.62	0.15	0.88	0.09	16.46
		Gastro-intestinal disease	0.51	0.61	-0.56	0.57	0.05	5.22
		Immunosuppression	0.69	0.38	-0.67	0.51	0.23	2.04

		Faecal incontinence	4.72	0.91	8.04	<0.001	3.23	6.89
		Urinary incontinence	0.77	0.55	-0.36	0.72	0.19	3.11
		Help provided by healthcare professional	1.15	0.39	0.41	0.68	0.59	2.22
		Help provided by family member	2.35	0.76	2.65	<0.001	1.25	4.42
		Antibiotic prescribed at hospital discharge	1.20	0.66	0.33	0.75	0.41	3.51
		Higher education	0.16	0.08	-3.45	<0.001	0.05	0.45
		Infection with ESBL during last hospitalisation	1.63	0.80	1.00	0.32	0.62	4.28
		Abdominal infection site	5.45	1.15	8.04	<0.001	3.61	8.25
	During follow-up	1 additional antibiotic course	2.13	1.70	0.95	0.34	0.45	10.15
		2 additional antibiotic courses	2.49	1.95	1.16	0.25	0.54	11.58
		3 additional antibiotic courses	5.90	2.35	4.47	<0.001	2.71	12.86
		Oral corticosteroids or other immunosuppressive drugs	0.52	0.48	-0.71	0.48	0.08	3.17
		Proton pump inhibitors	2.99	1.24	2.64	0.01	1.33	6.75
		H2-receptor antagonists	1.04	1.57	0.02	0.98	0.05	20.1
		Faecal incontinence	3.42	1.45	2.91	<0.001	1.49	7.84
		Urinary incontinence	1.36	1.05	0.40	0.69	0.30	6.17
		Indwelling device	3.94	2.91	1.86	0.06	0.93	16.76
		Urinary catheter	3.26	1.58	2.44	0.02	1.26	8.42
		Diarrhoea	1.60	0.63	1.21	0.23	0.74	3.45
		Autonomous	0.18	0.11	-2.88	<0.001	0.06	0.58
		Help provided by family member	3.97	1.83	3.00	<0.001	1.61	9.79
		Help provided by healthcare professional	3.00	1.86	1.77	0.08	0.89	10.12
		Help needed for dressing	1.44	1.14	0.46	0.65	0.30	6.82

		Help needed for urinary and faecal excretion	4.73	2.04	3.60	<0.001	2.03	11.01
		Help needed for food preparation	1.70	1.11	0.81	0.42	0.47	6.10
		Help needed for personal hygiene	1.23	0.98	0.26	0.79	0.26	5.84
		Help needed for medication intake	2.98	1.46	2.23	0.03	1.14	7.78
		Help needed for mobility	1.52	1.23	0.52	0.60	0.31	7.45
		Help needed for bed position shift	3.01	1.62	2.05	0.04	1.05	8.65
		Help needed for feeding	1.84	0.82	1.38	0.17	0.77	4.41
		≥ 2 hospitalisations after discharge	3.59	1.26	3.64	<0.001	1.80	7.15
		Spent time cooking meat products	0.53	0.19	-1.75	0.08	0.26	1.08
		Prepared food for other household members	0.53	0.20	-1.70	0.09	0.26	1.10
		Shared bath towels with other contacts	1.13	0.39	0.35	0.72	0.57	2.24
Household member	**Baseline**	Age	1.06	0.28	0.23	0.82	0.64	1.76
		Current antibiotic intake	3.86	4.14	1.26	0.21	0.47	31.59
		Higher education	0.94	0.48	-0.11	0.91	0.35	2.53
		Spouse of index case	3.65	1.50	3.14	<0.001	1.63	8.19
	Follow-up	Antibiotic intake	2.59	0.48	5.11	<0.001	1.80	3.73
		Proton pump inhibitors	2.96	1.34	2.39	0.02	1.21	7.20
		Active helper of index case	3.84	1.75	2.95	<0.001	1.57	9.39
		Spent time cooking meat products	1.11	0.71	0.17	0.87	0.32	3.90
		Prepare food for other household members	1.78	1.08	0.94	0.35	0.54	5.87
		Shared towel with index case	1.10	0.28	0.38	0.71	0.67	1.82

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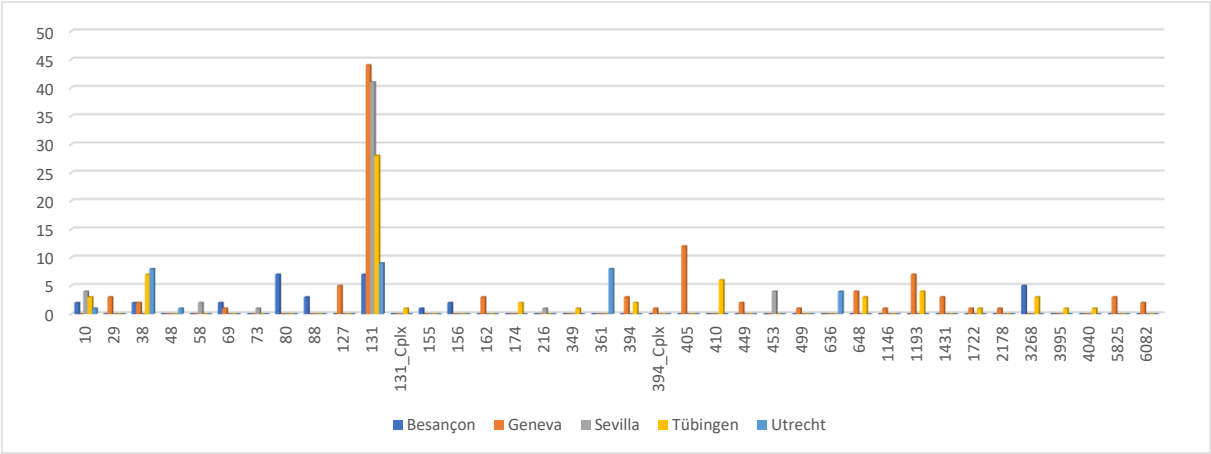
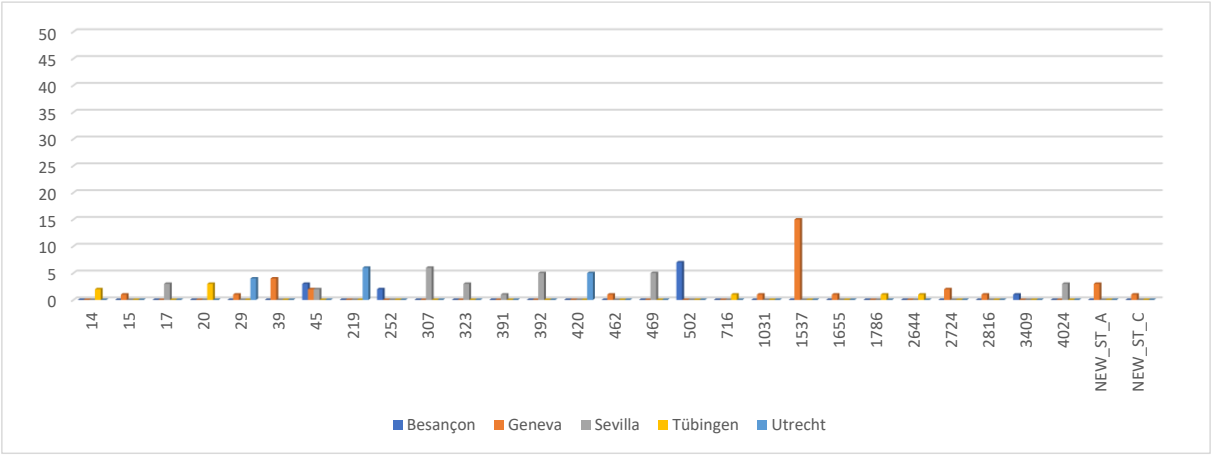
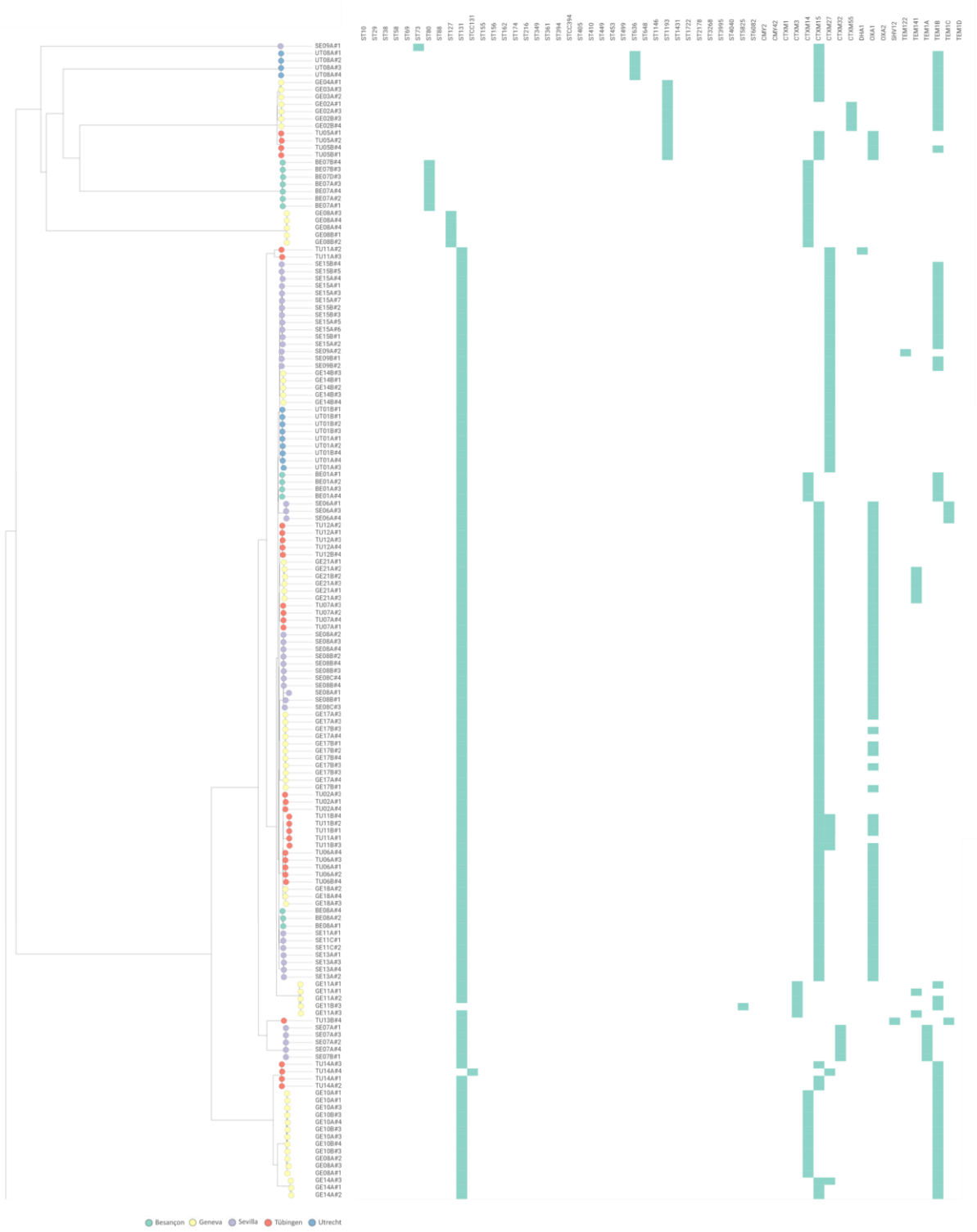
Supplementary Figure 1. Sequence type distribution of ESBL-producing E. coli isolates per centre. Two new MLST were identified in Geneva (belonging to the clonal complex CC394) and Tübingen (belonging to CC131).



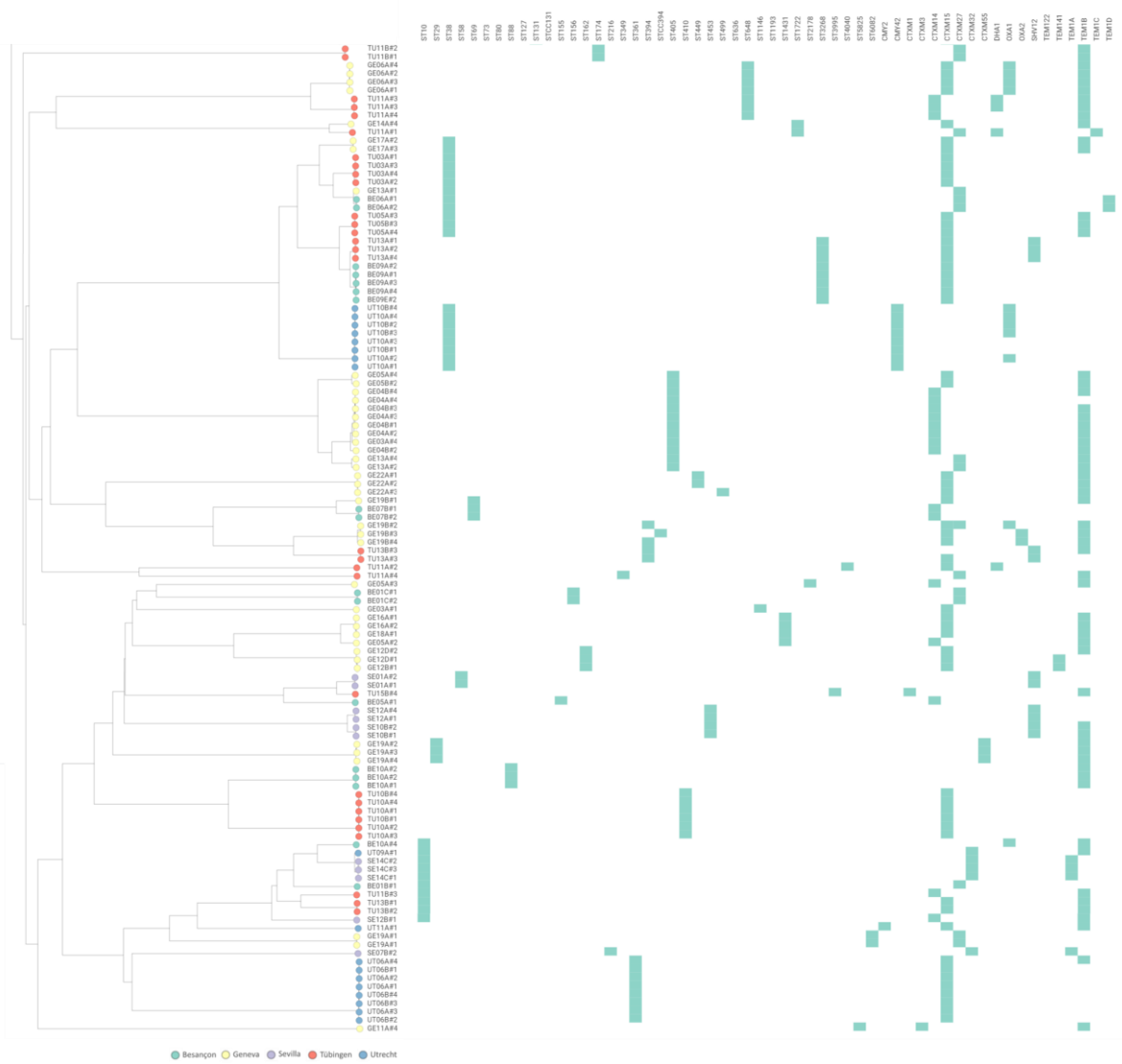
Supplementary Figure 2. Sequence type distribution of ESBL-producing K. pneumoniae isolates per centre. Two new MLST profiles were described in Geneva, named New-ST-A and New-ST-C.



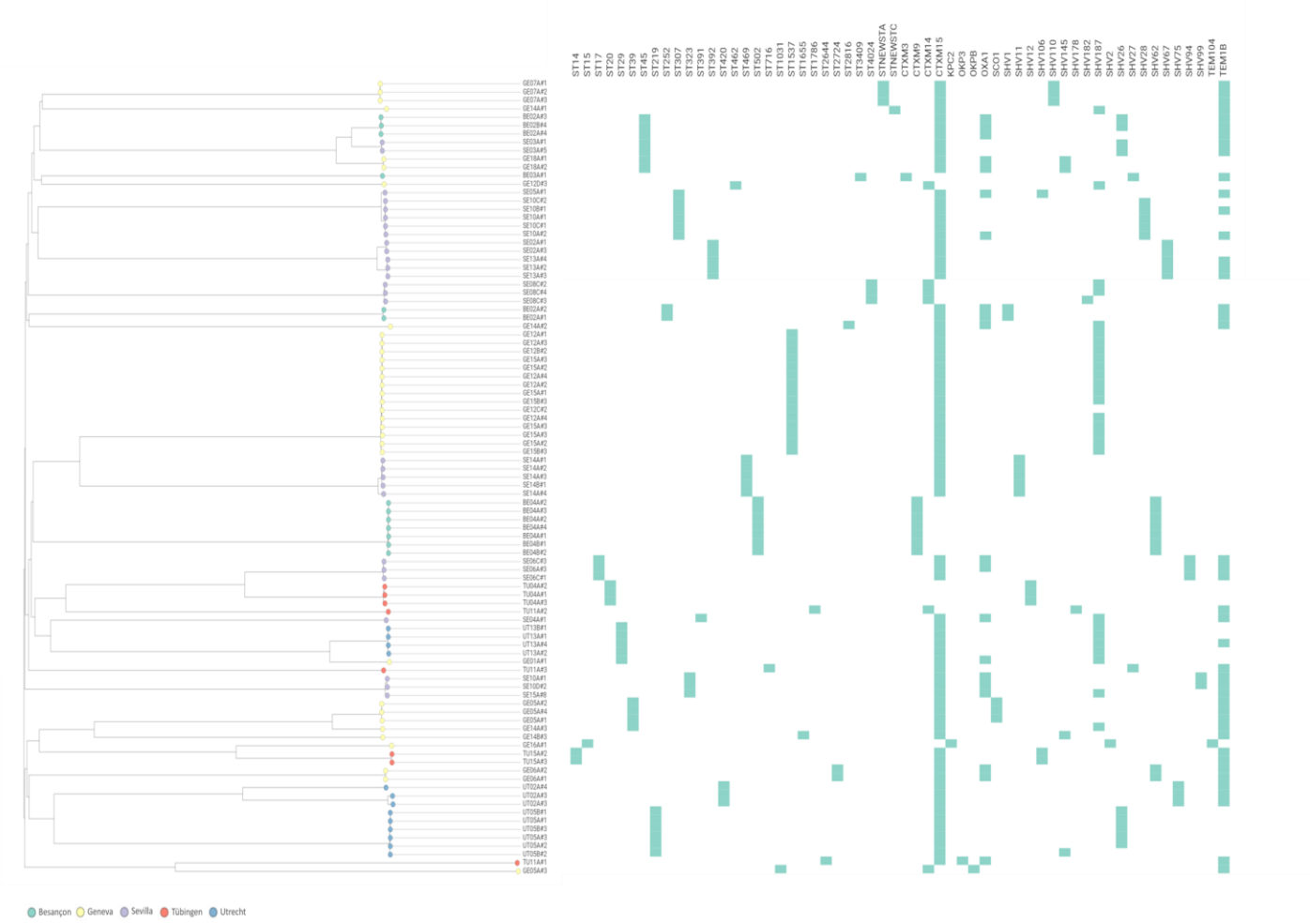
Supplementary Figure 3. Neighbour joining core genome phylogenetic tree of ESBL-producing E. coli isolates collected during the 4-month follow-up in the 5 study centres, constructed with SeqSphere+ using the Enterobase scheme. Colour code indicates the respective MLSTs (see the legend for details).



Supplementary Figure 3. (cont.)



Supplementary Figure 4. Neighbour joining core genome phylogenetic tree of ESBL-producing K. pneumoniae isolates collected during the 4-month follow-up in the 5 study centres , constructed with SeqSphere+ using the Enterobase scheme. Colour code indicates the respective MLST (see the legend for details).



CHAPTER THREE

Screening strategies and infection control measures to control nosocomial ESBL-PE and CPE

Part 1) Nation-wide survey of screening practices to detect carriers of multi-drug resistant organisms upon admission to Swiss healthcare institutions

A similar version of this chapter was published under the following reference:

Martischang R, Buetti N, Balmelli C, Saam M, Widmer A, Harbarth S. Nationwide survey of screening practices to detect carriers of multi-drug resistant organisms upon admission to Swiss healthcare institutions. Antimicrob Resist & Infect Control. 2019;8(37). DOI: 10.1186/s13756-019-0479-5.

Abstract

As emergence and spread of multi-drug resistant organisms (MDRO) requires a standardized preventive approach, we aimed to evaluate current MDRO admission screening practices in Swiss hospitals and to identify potential barriers impeding their implementation.

In early 2018, all Swiss public and private healthcare institutions providing inpatient care were contacted with a 34-item questionnaire to investigate current MDRO admission screening policies. Among 139 respondents representing 180 institutions (response rate, 79%), 83% (149) of institutions implemented MDRO admission screening, while 28% of private and 9% of public institutions did not perform any screening. Targeted high-risk screening included carbapenemase producers, extended-spectrum beta-lactamase producers and methicillin-resistant *Staphylococcus aureus* at the institutional level for respectively 78 % (115), 81 % (118) and 98 % (145) of screening institutions. Vancomycin-resistant enterococci (44 % of institutions), multi-resistant *Acinetobacter baumanii* (41 %) and *Pseudomonas aeruginosa* (37 %) were systematically searched only by a minority of screening institutions. A large diversity of risk factors for targeted screening and some heterogeneity in body sites screened were also observed. Admission-screening practices were mostly impeded by a difficulty to identify high-risk patients (44 %) and non-compliance of healthcare workers (35 %).

Heterogeneous practices and gaps in small and privately-owned institutions, as well as a mismatch between current epidemiologic MDRO trends and screening practices were noticed. These results highlight the need for uniform national MDRO screening standards.

Introduction

Early detection of multi-drug resistant organisms (MDRO) carriage upon admission could allow timely implementation of infection control measures and the appropriate selection of empiric antimicrobial therapy.(1) Few nationwide surveys investigated real-life MDRO screening practices upon admission.(2–5) In 2010, an unpublished survey conducted in Swiss intensive care units (ICUs) revealed heterogeneous MDRO screening practices. Endemicity among MDROs in Switzerland differs according to community or hospital settings. ESBL-producing *Escherichia coli* is considered as endemic in the general population, especially in the institutionalized elderly (ESBL *E.coli* prevalence of 22% among clinical isolates from nursing homes in 2017)(6), whereas acute care hospitals also consider MRSA - despite decreasing trends - (prevalence of 8% among clinical *S. aureus* isolates in 2014)(7) and ESBL-producing *Klebsiella pneumoniae* as endemic (7.7% of ESC-R invasive isolates in 2017).(8) The emergence and spread of MDRO requires a standardized preventive approach on a national scale.

Specific aims

We therefore evaluated current MDRO admission screening practices in Swiss hospitals and identified potential barriers impeding their implementation.

Methods

From January to March 2018, a nation-wide 34-item questionnaire was sent to 228 Swiss public and private healthcare institutions providing inpatient acute care. Psychiatric institutions, nursing homes, palliative

care and pain therapy centers were excluded. Three reminders as well as a phone call were addressed to each non-responding institution.

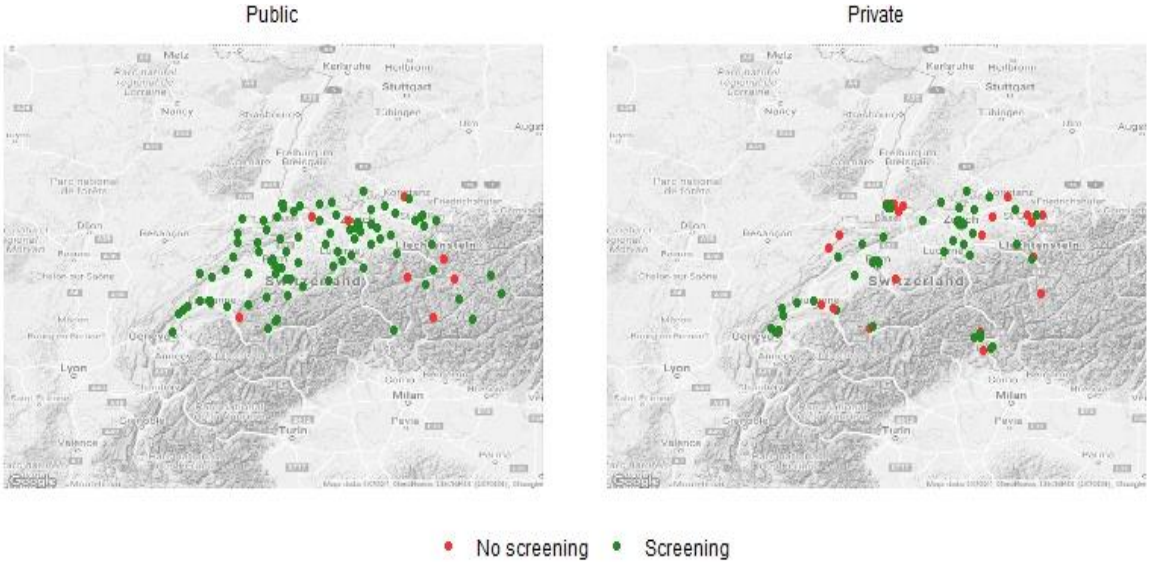
The survey was translated in the three official languages, pre-tested locally and shared through the online platform SurveyMonkey® (see French and German versions of the Online Survey, additional file 1 and 2). We collected information about the characteristics of each hospital, in addition to current practices concerning universal and targeted MDRO screening for patients at-risk at admission, risk factors considered for targeted screening, body sites for sampling swabs and cultures, preemptive contact precautions for high-risk patients, the presence of local guidelines and problems faced to implement on-admission screening.

All analyses were institution-based (n=180) and not respondent-based (n=139), since some respondents were in charge of several institutions. Data were extracted from the online platform to an Excel® spread-sheet, checked for accuracy and exported for descriptive analysis using RStudio and STATA 15.0® (StataCorp LLC, College Station, TX).

Results

Overall, 139 respondents, mainly nurses (56%) and physicians (37%) replied for 180 institutions (response rate, 79%), with 57 % from public institutions and 61 % from small-size (< 200 beds), 21 % medium-size, and 18 % large-size institutions (> 500 beds). All non-responders were small-size institutions. The majority of hospitals (72%) was located in the Swiss-German part. Eighty-three percent of institutions (149) implemented some type of MDRO admission screening, while 28% of private and 9% of public institutions did not perform any screening (Figure 1).

Figure 1. Implementation of admission screening for at least one MDRO among public and private institutions



Universal methicillin-resistant *Staphylococcus aureus* (MRSA) screening of all admitted patients was not performed on an institutional level by any hospital, except for a few specific units in 6% of screening institutions. Targeted high-risk screening at the institutional level included carbapenemase-producing *Enterobacteriaceae* (CPE), extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* and MRSA, which were monitored by 78 % (n=115), 81 % (n=118) and 98 % (n=145) of hospitals, respectively (Table 1, Figure 2 & 3).

Figure 2. Implementation of admission screening for ESBL-PE among public and private institutions

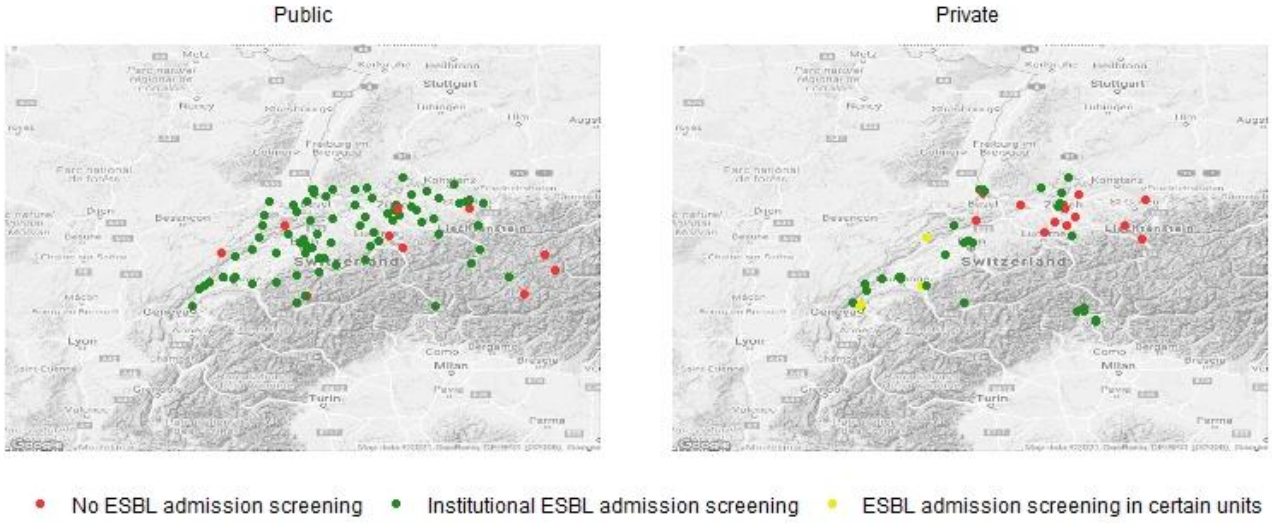
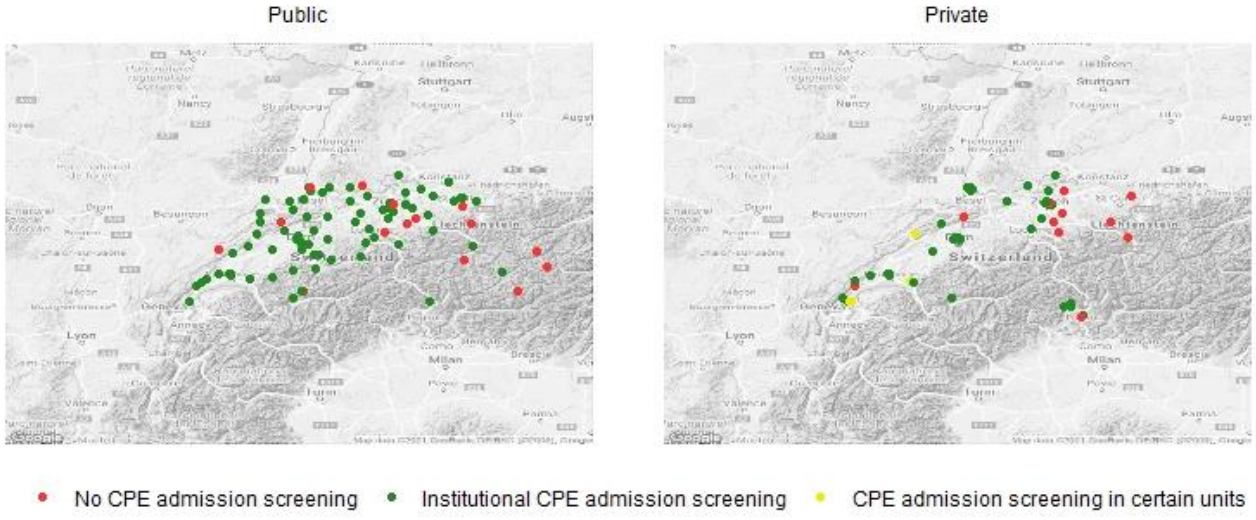


Figure 3. Implementation of admission screening for CPE among public and private institutions



Vancomycin-resistant enterococci (VRE) (44%), multi-resistant *Acinetobacter baumanii* (41%) and *Pseudomonas aeruginosa* (37%) were systematically searched only by a minority of institutions with on-admission screening programs, without differences between small and large institutions.

Table 1. Targeted high-risk MDRO screening among public and private hospitals in Switzerland

	ESBL	CPE	MDR-*Acinetobacter*	MDR-*Pseudomonas*	VRE	MRSA
Targeted screening (%)						
Public (n=102)[1]:						
Institutional:	82 (89%)	77 (83%)	37 (40%)	36 (39%)	38 (41%)	93(100%)
Only in certain units:	0	0	1 (1%)	2 (2%)	4 (4%)	0
None:	10 (11%)	16 (17%)	55 (59%)	55 (59%)	51 (55%)	0
Private (n=78)[2]:						
Institutional:	36 (67%)	38 (70%)	23 (43%)	18 (33%)	27 (50%)	52 (95%)
Only in certain units:	4 (7%)	4 (8%)	2 (4%)	8 (15%)	3 (6%)	3 (5%)
None:	14 (26%)	12 (22%)	28 (53%)	28 (52%)	24 (44%)	0

Abbreviations:

ESBL: Extended-spectrum beta-lactamase

CPE: Carbapenemase-producing Enterobacteriaceae

MDR: Multi-Drug Resistant

VRE: Vancomycin Resistant Enterococcus

MRSA: Methicillin Resistant Staphylococcus aureus

Footnote to Table 1:

[1]Missing values for: ESBL = 10, CPE= 9, Acinetobacter baumanii = 9, Pseudomonas aeruginosa = 9, VRE = 9 and MRSA= 9.

[2] Missing values for: ESBL= 24, CPE= 24, Acinetobacter baumanii = 25, Pseudomonas aeruginosa = 24, VRE = 24 and MRSA= 23.

Frequently used risk factors to screen patients considered at high risk for MDRO carriage were "known carriers", "hospitalization abroad" and a "direct transfer from abroad" (Table 2). Other risk factors are heterogeneously recognized among institutions. Of note, few hospitals (19%) systematically screen patients who have been transferred from other Swiss hospitals for VRE carriage, despite increasing VRE rates and ongoing outbreaks in Switzerland.

Table 2. Patient-level risk factors considered for targeted MDRO screening upon admission

	ESBL (n=122)	CPE (n=119)	MDR-Acinetobacter (n=62)[1]	MDR-Pseudomonas (n=63)[1]	VRE (n=72)	MRSA (n=148)
	(n = number of centers performing a targeted screening for each pathogen)					
Risk factors used for targeted admission screening (%)						
Known MDRO patient:	111 (91%)	111 (93%)	59 (95%)	60 (95%)	67 (93%)	143 (97%)
Direct transfer from abroad:	114 (93%)	107 (90%)	41 (66%)	37 (59%)	54 (75%)	144 (97%)
Direct transfer from Switzerland[2]:	33 (27%)	29 (24%)	13 (21%)	14 (22%)	14 (19%)	71 (48%)
Transfer from a long term care facility:	11 (9%)	7 (6%)	3 (5%)	4 (6%)	5 (7%)	32 (22%)
Hospitalization abroad in the recent past[3]:	103 (84%)	98 (82%)	37 (59%)	32 (51%)	47 (65%)	109 (74%)
Travel in a country with endemic MDRO:	28 (23%)	34 (29%)	16 (25%)	18 (29%)	19 (26%)	35 (24%)
Other:	38 (31%)	41 (34%)	23 (37%)	21 (33%)	21 (29%)	84 (57%)

Abbreviations:

ESBL: Extended-spectrum beta-lactamase

CPE: Carbapenemase-producing Enterobacteriaceae

MDR: Multi-Drug Resistant

VRE: Vancomycin Resistant Enterococcus

MRSA: Methicillin Resistant Staphylococcus aureus

Footnote to Table 2:

[1] *Missing values for: MDR Acinetobacter baumanii = 1, MDR Pseudomonas aeruginosa = 1.*

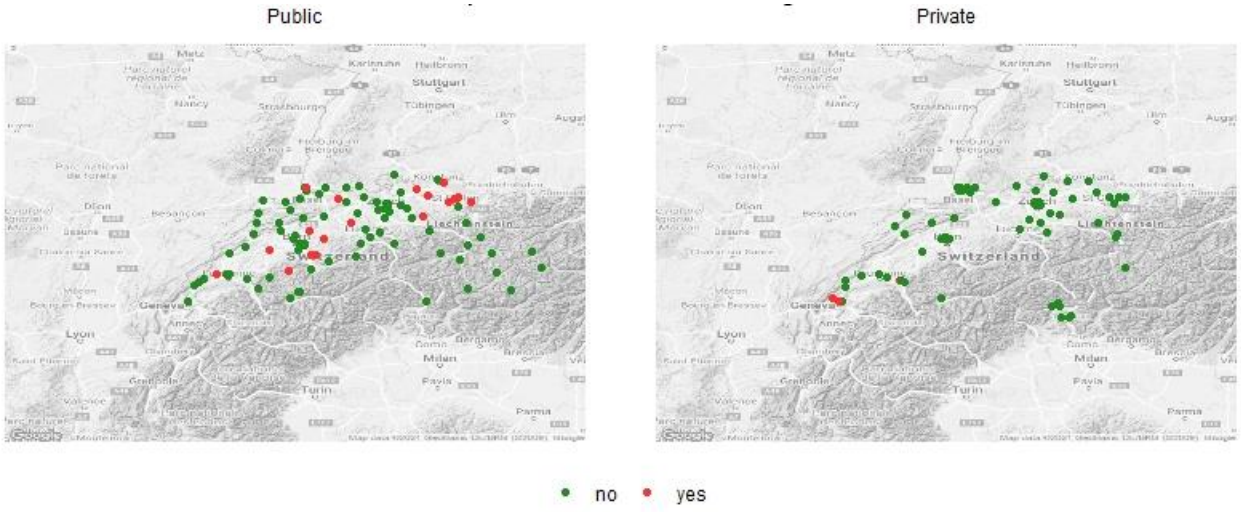
[2] *Mainly West Switzerland and Tessin were targeted when considering a direct transfer from Switzerland.*

[3] *Varying timeframes considered as recent past, mainly from 6 to 12 months.*

Heterogeneity subsists on the choice of body site sampling. Nares (99%), throat (81%) and inguinal sampling (91%) are leading body sites to screen for MRSA, whereas anal or rectal swabs are most frequently used for ESBL (89%), CPE (94%) or VRE (88%) screening. However, in some centers, inguinal screening was also performed for enteric bacteria. For MDR-*A. baumanii* and *P. aeruginosa*, a large variety of body sites were screened (anal, rectal, inguinal, throat or nasal swabs). For high-risk patients, only 23% (33/142) of hospitals routinely performed repeat swabs in case of one negative screening result. A total of 90% (86/96) of ICUs implemented pre-emptive contact precautions, including placement in a single room in 63% of ICUs.

Despite local recommendations for admission screening provided by 96% (137/142) of hospitals, these practices were mostly impeded by a difficulty to identify high-risk patients (44%) and non-compliance of healthcare workers (35%). Reimbursement issues were less commonly cited as an obstacle (15%) and was predominant in public institutions (Figure 4).

Figure 4. Reimbursement cited as a barrier to implement MDRO screening



Discussion

This nation-wide survey to examine current practices of MDRO admission screening was answered by 180 institutions, representing an excellent response rate and the diversity of healthcare institutions in Switzerland, among public and private institutions of different sizes. This survey revealed good compliance with on-admission MDRO screening practices in larger acute-care hospitals, but also important gaps in small and private institutions.

This survey differs from previous national surveys evaluating MDRO screening practices at admission, mainly because of its higher response rate and the reporting of both risk factors and body sites sampled according to MDRO species.(2–5) Only one national survey performed in France in 2012 addressed public and private healthcare facilities. This survey observed that only 34% of 286 institutions reported management of patients at-risk at the time of admission.(3)

A mismatch between the current epidemiologic situation and screening practices was noticed with a disproportionate focus on MRSA (in particular

in patients transferred from the French and Italian speaking parts of Switzerland) and a lack of awareness of possible spread of *A. baumanii*, *P. aeruginosa* and VRE by unknown carriers, including patients transferred within Switzerland. Indeed, nosocomial MRSA incidence has been declining, whereas VRE rates are rapidly increasing.(7,9,10) In addition, severe nosocomial outbreaks of *A. baumanii* infections linked to imported cases have occurred in Switzerland in the past.(11) Therefore, targeted high-risk screening should also include other MDROs beside MRSA.

A recent travel history to foreign countries without hospitalisation was rarely used as a risk factor to define high-risk patients eligible for screening at admission (23-29% of institutions according to the type of MDRO). This policy concerned in particular South-Asian countries with hyperendemic MDRO occurrence, such as India, Pakistan, Bangladesh, Nepal and Sri Lanka. A recent travel history to North America or U.S. citizenship were not considered as risk factors by any Swiss institution, despite increasing importation of community MRSA into Switzerland.(12)

Heterogeneity was also observed among risk factors considered for targeted screening, probably due to a lack of national consensus on multiple criteria supporting surveillance programs. Adding to this complexity, actual controversies addressing admission screening policies support the requirement for updated and uniform standards: species to be screened, risk factors considered for targeted screening, number of screening swabs to be performed at admission, among others. Interestingly, cost considerations did not play an important role in implementing MDRO screening policies.

This survey has limitations. First, we were unable to perform external validation of the respondents' answers. Second, this survey did cover

neither screening practices beyond the admission procedure nor variability in MDRO control measures or laboratory detection methods. Third, the design of the study did not allow correlating MDRO screening practices to nosocomial MDRO transmission rates.

Conclusions

In summary, these results highlight the need for uniform national MDRO screening standards. It also demonstrates a lack of awareness about current MDRO trends, focusing on MRSA rather than VRE or gram-negative MDROs, and ongoing confusion about risk factors that might be addressed through uniform national standards. Harmonized, clear and accessible guidelines – which are already available in some countries – could support standardization of risk factors used for targeted admission screening and of sample sites for admission screening.(13,14)

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Part 1) First reported nosocomial outbreak of NDM-1 producing *Escherichia coli* in Switzerland

A similar version of this chapter was published under the following reference:

Martischang R, Chraiti M-N, Lazarevic V, Gaia N, Bandiera-Clerc C, Soule H, Renzi G, Iten A, Ginet C, Pittet D, Schrenzel J, Harbarth S. First reported nosocomial outbreak of NDM-1 producing Escherichia coli in Switzerland. International Conference on Prevention and Infection Control, Geneva, September 2019. Oral presentation # O45

Introduction

Since 2008, NDM-producing Enterobacteriaceae has spread globally. In late 2017, a patient transferred from Dubai was identified as NDM-producing E.coli carrier, and placed under contact precautions during two hospital stays at HUG in Jan and Jul 2018. Between Nov 2018 and May 2019, 3 secondary cases who had not travelled outside Switzerland for the past 12 months were found colonized with NDM-producing E. coli by routine screening swabs or urine cultures. Nosocomial cross-transmission was strongly suspected.

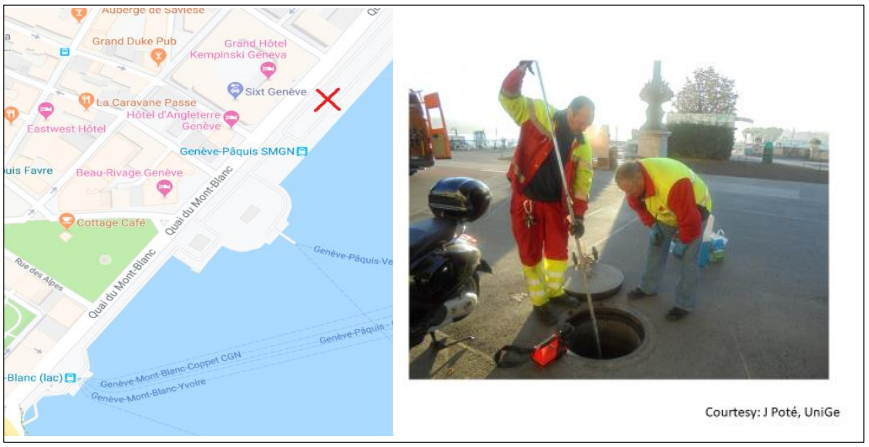
Objectives

We report an outbreak investigation guided through molecular approaches.

Methods

Roommates' screening (July and Nov 18, May 19), and environmental screening and disinfection (May 19) in the concerned patient room were performed. Following Illumina iSeq sequencing, the relatedness between 4 NDM isolates was assessed by cgMLST and cgSNP analyses. Additional environmental Enterobacterales strains originating from sewage in Geneva (Figure 1) were included to the scheme to evaluate potential community dissemination.

Figure 1. Environmental sampling in Geneva sewage

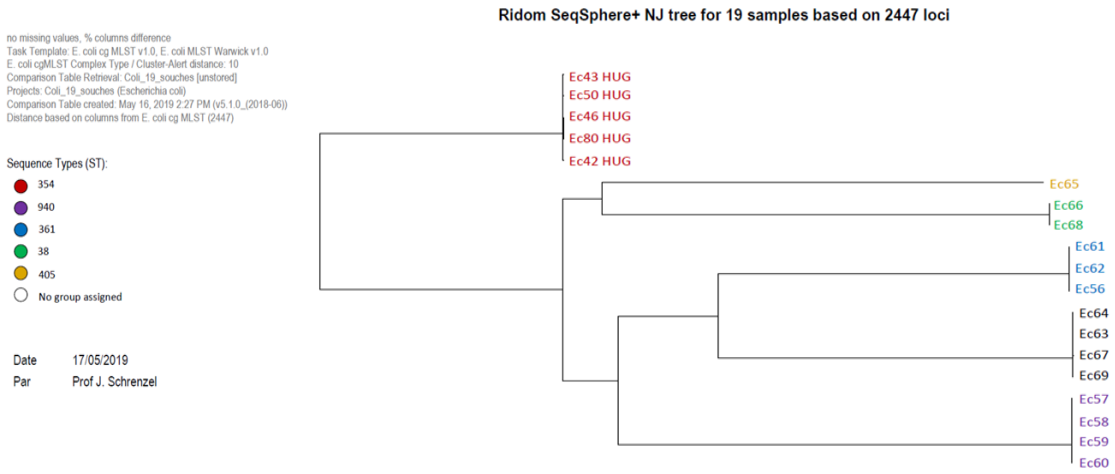


Results

Spatiotemporal analyses identified the simultaneous passage of 2 patients in a newly opened surgical step-down unit in July 18, and staggered passage of 3 patients in the same room on a private floor from Nov 18 through Apr 19. As of today (May 25), 20 environmental samples

and all further contact screening swabs have been negative. Sequencing analysis confirmed cross-transmission with E. coli ST354 NDM-1 (<10SNPs). No relatedness was observed with community strains. Standard precautions were reinforced in the concerned units. We implemented a computerized readmission alert system of all contact patients with potential exposition, requiring mandatory screening at re-admission. One of the patients died of surgical complications unrelated to E. coli NDM-1 carriage.

Figure 2. Phylogenetic tree of NDM-producing E.coli originating from Geneva University Hospitals (Ec 42,43,46,50,80) and from community environment (Other Ec).



Conclusions

To our knowledge, this cluster represents the first nosocomial NDM-producing *E. coli* outbreak in Switzerland, despite implementation of strict contact precautions for the index case. The fortuitous detection of cases by the weekly universal screening implemented in intensive care units facilitate early control of this prolonged institutional outbreak. The retrieved *E. coli* ST354 clone has so far mostly been reported from animals, and was rarely associated with carbapenemases. This outbreak confirms the high nosocomial transmission potential of these highly resistant Enterobacteriaceae.

Part 2) An interventional quasi-experimental study to evaluate the impact of a rapid screening strategy in improving control of nosocomial extended-spectrum beta-lactamase producing Enterobacterales and carbapenemase-producing organisms in critically ill patients

MS in preparation (presented in International Conference on Prevention and Infection Control. 2021. Oral presentation # O02.).

Abstract

Introduction

Rapid molecular tests could accelerate the surveillance and control of extended-spectrum beta-lactamase producing Enterobacterales (ESBL-PE) and Carbapenemases-Producing Organisms (CPO) in intensive care units (ICUs).

Objective and Methods

This interventional 12-month cohort study compared a loop-mediated isothermal amplification (LAMP) assay performed directly on rectal swabs with traditional culturing methods (control period, 6 months), for targeted screening at admission and universal weekly screening of all ICU patients. Contact precautions (CP) were implemented for all carriers of CPO or non-*E.coli* ESBL-producing Enterobacterales (nEcESBL-PE). Using survival analysis, we compared the median time intervals from admission to discontinuation of unnecessary preemptive CP among patients at risk and the median time intervals from screening to implementation of CP among newly identified carriers. We also compared diagnostic performances, and nEcESBL-PE/CPO acquisition rates. This study is registered, ISRCTN 23588440.

Results

We included 1’043 patients (median length of ICU stay, 2.2 days). During the intervention and control phases, 92/147 and 47/86 of patients at-risk screened at admission were candidates for early discontinuation of preemptive CP. Similarly, 16/589 and 4/313 newly discovered carriers by weekly screening were candidates for implementation of CPs. The LAMP assay had a positive predictive value (PPV) of 44.0% and negative predictive value (NPV) of 99.9% for CPO, and 55.6% PPV and 98.2% NPV

for nEcESBL-PE. The median time from admission to discontinuation of preemptive CP increased during the interventional period from 80.5 (95%CI 71.5-132.1) to 88.3 (95%CI 57.7-103.7) hours (p=0.47). Due to the poor PPV, we had to stop using the LAMP assay to implement CP. Compared to the control period, the incidence rate ratios for nEcESBL-PE and CPO acquisition during the intervention phase were 0.80 [95%CI 0.36-1.75] and 0.23 [95%CI 0.03-1.76] after adjustment for colonization pressure and hand hygiene compliance.

Conclusion

A rapid screening strategy with LAMP assays performed directly on rectal swabs had no benefit for infection control in a low-endemicity setting. This study highlights the limitations and challenges of molecular screening tests and their routine use in the ICU setting.

Introduction

Digestive carriage of extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-PE) and carbapenemase-producing organisms (CPO) places patients at risk of antibiotic-resistant infection, increasing length of hospital stay(137,138) and mortality.(1,138) Active surveillance as part of a multimodel approach already proved to be efficient to decrease ESBL and CPO infections.(54,139) In intensive care units (ICUs), admission and weekly universal screenings may help to detect new CPO and ESBL-PE carriers. In case of patients at risk, preemptive contact precautions (CP) after admission may be discontinued after negative results. However, current microbiologic screening methods are slow, delaying the discontinuation of preemptive CP, with possible harmful effects.(140),(141) This diagnostic delay also impacts detection of previously unknown carriers screened during routine surveillance, leading to an increased risk of cross-transmission.

Molecular screening methods such as loop-mediated isothermal amplification (LAMP) reaction tests have been developed to improve diagnostic performance(111) and accelerate the slow turn-around observed with traditional culture-based systems.(112,113,114) However, several reviews recently stressed the lack of clinical effectiveness studies.(142,143) In order to improve CPO and ESBL-PE control in the ICU setting, LAMP tests may yet represent a reasonably fast and specific, but also cost-effective screening method.(117) We hypothesized that a rapid LAMP assay performed directly on rectal swabs could yield individual and ecological benefits compared to traditional phenotypic methods, accelerating the discontinuation of unnecessary preemptive CP for negative patients screened at admission, and the implementation of CP for newly identified carriers, reducing ESBL-PE and CPO incidence among critically ill patients.

Material and methods

Setting and population

Geneva University Hospitals is a tertiary care center with 36 ICU beds. The mixed medical-surgical ICU admits 2'500 patients per year with an median length of stay of 1.9 days. The mean weekly prevalence of ESBL-PE carriage was 10.2% in 2016, with an average of 2.4 newly identified ESBL-PE positive patients per week.

All ICU patients with a surveillance screening for ESBL-PE and CPO by rectal swabs or stool cultures were included in this study. The impact of rapid screening tests on de-implementation of preemptive CP was evaluated on a first sub-group of patients at risk of ESBL-PE and CPO carriage, screened at admission. It was further evaluated on a second sub-group of patients screened weekly during ICU stay. Patients with a competing and microbiologically proven indication for CP were excluded.

Study design

This prospective, interventional, quasi-experimental cohort study compared a rapid molecular test (LAMP assay) performed directly on all rectal swabs during a first 12-month intervention period (April 2019-March 2020) with conventional culturing methods during a second 6-month control period (May-October 2020) after a one-month wash-out period.

Outcomes and definitions

The primary outcome was the median time interval from admission to discontinuation of unnecessary preemptive CP among patients at risk screened upon ICU admission. Secondary outcomes included the median time from screening to implementation of CP among newly identified carriers, laboratory turn-around-times (TATs), diagnostic performances

and ICU-acquired non-*E.coli* ESBL-producing Enterobacterales (nEcESBL-PE) or CPO acquisition events, defined as a newly detected nEcESBL-PE or CPO carriers by screening or clinical culture. Incidence rates of nEcESBL-PE and CPO acquisition were defined per 1'000 patient-days at risk.

Surveillance screening and infection control measures

Admission screening targeted patients with specific risk profiles, including also a subpopulation of patients considered at high risk according to their prior exposure history (Suppl. Table 1). Weekly universal screening was performed for all ICU patients present on Monday morning. Additional screening of roommates was performed for active case finding in case of cluster investigations.

nEcESBL-PE or CPO carriers were identified by door signage, flagged using automatized alert systems, and placed under CP, which included dedicated material (gowns, gloves), spatial separation, and environmental decontamination. Preemptive CP were discontinued at the first negative result for patients at-risk, or after sequential screenings for patients at high risk (e.g. previously known CPO carrier). Microbiological results were actively screened by dedicated infection control nurses, to ensure adequate discontinuation or implementation of infection control measures. Timing and adequacy of prescription for screening and CP were monitored by a dedicated nurse.

Microbiological procedures

Routine screening procedures with conventional culture methods

Rectal swabs (eSwab™, Copan) were routinely collected by trained ICU nurses. Swabs were then plated on three media: chromID ESBL

(bioMérieux), , chromID® OXA-48 (bioMérieux), and CHROMagar™ *Acinetobacter* (CHROMagar, France). All colonies with specific colors according to manufacturers' instructions were identified by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry and the antibiotic susceptibility profile of each isolate was determined by disc diffusion method using EUCAST recommendations.(144) For ESBL confirmation, we used double-disk synergy tests. In doubtful cases, ESBL + AmpC Screen Kit 98008 (Rosco Diagnostica) were used as a second line confirmatory test. For CPO confirmation, we used the LAMP eazyplex® SuperBug CRE system (AxonLab, UK) on selected isolates, a qualitative molecular test covering CTX M-1 and CTX M-9 families, KPC variants (KPC2 to KPC15), NDM variants (NDM1 to NDM7), VIM variants (VIM1 to VIM37), OXA-48-like variants (OXA-48, OXA-162, OXA-204, and OXA-244), and OXA-181-like variants (OXA-181, and OXA-232).(145)

Workflow

The bacteriology laboratory processed non-stop all diagnostic samples related to the study during weekdays until 17h00. Of note, plating of isolates, incubation, and culture triage were automatized from March 2019 onwards.(146)

Interventional screening strategy (LAMP assay)

Rectal swabs were split into three equal parts and processed simultaneously. A first part was run by LAMP eazyplex® SuperBug CRE system as described above to detect the main genes coding for ESBLs and carbapenemases. A second part was processed using standard bacteriology methods for pathogen identification and quality assurance purposes (i.e. confirmed presence of *E.coli*). A third part was stored at -20°C to resolve any potential discordant results between molecular and

phenotypic approaches. The results were communicated in real-time from Monday to Friday.

Unnecessary preemptive CP were stopped based on negative LAMP results, and CP were implemented for newly identified patients based on LAMP-positive results for CPO, or culture-positive results for nEcESBL-PE. Cultures were used as the reference test in case of discordant results. LAMP-positive and culture-negative samples were investigated post-hoc using specific PCRs (TEM, SHV). Isolates from samples negative by LAMP were retested using LAMP and disk diffusion methods.

Unnecessary preemptive CP were stopped based on negative LAMP results, and CP were implemented for newly identified patients based on LAMP-positive results for CPO, or culture-positive results for nEcESBL-PE. Cultures were used to inform infection control measures in case of discordant results. LAMP-positive and culture-negative samples were investigated post-hoc using specific PCRs (TEM, SHV). Isolates from samples negative by LAMP were retested using LAMP and disk diffusion methods.

Pilot study

This rapid testing strategy has been previously validated in our institution and showed high sensitivity and specificity.(147) In 2018, we included 209 samples from 187 ICU patients and observed a TAT gain of 44.1 hours with the LAMP technology compared to conventional methods, with a high specificity and negative predictive value (respectively 98.8% and 97.6%).(148) In the present study, the diagnostic performance of LAMP was again evaluated among all samples processed by both LAMP and cultures, the last being used as a reference test.

Data collection

TATs were categorized into pre-analytical TAT (time from admission to screening, time from screening to sample delivery to the laboratory), analytical TAT (time from arrival at the laboratory to reporting of results), and post-analytical TAT (time from result notification to implementation or discontinuation of CP). Pre-analytical and analytical TATs were collected from computerized laboratory databases. Post-analytical TAT was computed based on the date and time of implementation or discontinuation of CP, directly informed by the electronic patient file.

Acquisition events were collected using screening and clinical cultures from routine surveillance data. Colonization pressure was defined as the monthly sum of positive screening and clinical cultures for ESBL-PE and CPO. Only the first ESBL-PE or CPO isolate was considered per patient. Monthly hand hygiene compliance of healthcare workers was collected according to WHO methods. Systemic antibiotic consumption (ATC J01) was measured in daily doses per 1'000 patient-days. Adherence to screening and contact precautions was measured as defined in the Suppl. Appendix 1.

Statistical analysis

Time benefits for infection control

Analytical TATs expressed as medians were first compared using Wilcoxon rank-sum test, χ^2 test and Fisher exact test when appropriate. Unnecessary times (in days) spent under preemptive CP among patients screened at admission were compared for the intervention and control periods using survival analysis. Right censoring of patients occurred at ICU discharge or death, which were consequently regarded as competing events. Proportional subdistribution hazard modelling was performed in addition to cause-specific hazard models to account for competing events. Subdistribution hazard ratio (sHR) and cause-specific hazard

ratios (csHR) were calculated using Fine and Grey models, and Cox models, respectively. Hazards proportionality was tested by the visual examination of Schoenfeld residuals.

TATs were evaluated in an intention-to-treat analysis, regardless of the patient status (at-risk or at a high risk) and study-related laboratory activity, which was interrupted during weekends and public holidays. Several exploratory analyses were also performed. First, to estimate the effect of the rapid screening strategy on actionable results (without the need of sequential screening), we performed the same analysis, excluding patients screened during holidays and at high risk. Second, we reported detailed pre-analytical, analytical, and post-analytical TATs of patients screened at admission with CP discontinuation before discharge, excluding patients screened during holidays and at high risk.

Impact on nECESBL-PE and CPO acquisition rates

Chi-square or Fisher's exact tests were used to compare categorical variables and Student's t-test, for continuous variables. The impact of the interventional screening strategy on adjusted incidence-density ratios of nEcESBL-PE or CPO acquisition was compared using Poisson regression, accounting for aggregate-level exposures, including colonization pressure and hand hygiene compliance. All analyses were performed using R (version 4.0).

Ethical statement

This study was approved by the local Ethics Committee Review. It was considered as a quality improvement project, relying on routine surveillance data, and was therefore exempted from individual patient consent. This study is registered, ISRCTN 23588440.

Results

This study included 1'043 patients sampled 1'778 times (median length of stay, 2.2 days), including 231 patients with a targeted screening at admission and 896 patients with either weekly or epidemiologically indicated screening (Figure 1). Of 231 patients screened at admission, we distinguished 58 (25.1%) patients at high risk requiring sequential screening, and 173 (75.0%) patients at risk (Table 1). Most patients at-risk were transferred from another hospital (53.2%), and most patients at a high risk were already known carriers (46.6%, Suppl. Appendix 2). Among all patients screened at admission, 185 (80.1%) had preemptive CP. Among them¸ only 12 (6.5%) were positive for ncEC-ESBLPE or CPO, and 34 (18.4%) had an alternative indication to maintain CP, including known carriage of other MDROs. Thus, 139 (75.1%) patients screened at admission were included in the final analysis (Fig. 1). As shown in Fig. 1, 42 (4.7%) patients screened routinely during ICU stay were positive by either LAMP or culture for either CPO or ESBL-producing non-*E.coli*. After exclusion of 22 patients with known carriage or competing indication for CP, 20 patients were included in the final analysis.

Figure 1. Flowchart of included ICU patients

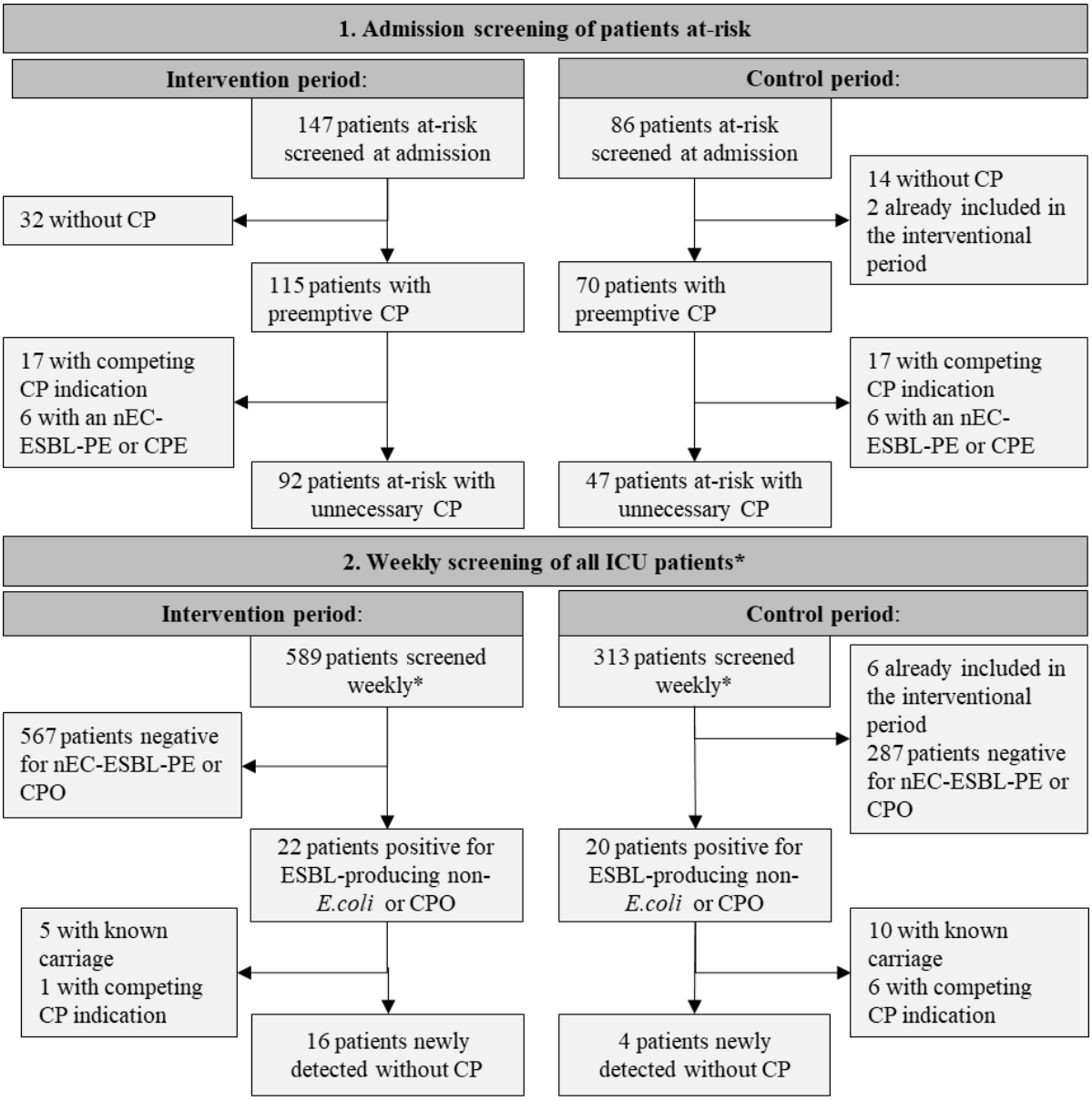


Table 1. Individual and aggregated characteristics of study participants and ICU patients.

	Interventional period	**Control period**	
Among participants screened at admissions	**n= 147 patients[a]**	**N= 86 patients[a]**	**P-value**
Patients at a high risk (%)	30 (20.4%)	29 (33.7%)	**0.04**
Median length of stay (days, IQR)	2.8 (1.5-5.6)	2.8 (1.7-7.1)	0.67
nEcESBL-PE carriers	10 (6.8%)	8 (9.3%)	0.68
CPO carriers	1 (0.7 %)	3 (3.5%)	0.14
CPE carriers	1 (0.7 %)	3 (3.5%)	0.14
Preemptive contact precautions prescribed	115 (78.2%)	70 (81.4%)	0.45
Among all participants screened weekly	**n= 589 patients[b]**	**n= 313 patients[b]**	**P-value**
Median length of stay (days, IQR)[c]	6.9 (2.9-12.6)	6.9 (3.8-15.1)	0.06
nEcESBL-PE carriers	19 (3.2%)	16 (5.1%)	0.22
CPO carriers	4 (0.7%)	8 (2.6%)	0.03
CPE carriers	3 (0.5%)	3 (1.0%)	0.42
Newly detected carriers	13 (2.2%)	10 (3.2%)	0.52
Contact precautions implemented at the time of detection among newly detected carriers	1/13 (7.7%)	6/10 (60%)	0.02
Colonization pressure (incidence density)	**n=8'884 patient-days**	**n=3'772 patients-days**	**P-value**
ESBL-PE (cases per 1'000 patient days)	15.3 [95%CI 12.8-18.1]	19.1 [95%CI 14.9-24.0]	0.13
CPO (cases per 1'000 patient days)	0.7 [95%CI 0.2-1.5]	2.9 [95%CI 1.5-5.2]	0.05
CPE (cases per 1'000 patient days)	0.4 [95%CI 0.1-1.1]	1.6 [95%CI 0.6-3.5]	0.22
Antibiotic consumption (ATC JC01)	**n=8'884 patient-days**	**n=3'772 patients-days**	**P-value**
Mean monthly consumption (DDD per 1'000 patient days, 95%CI)	813 [95%CI 722-899]	728 [95%CI 471-986]	0.48

Hand hygiene compliance among healthcare workers	Hand hygiene opportunities = 474	Hand hygiene opportunities = 360	P-value
Pooled mean of hand hygiene compliance	59.7 [95%CI 55.3-64.1]	61.1 [95%CI 56.1-66.1]	0.73

Footnote to table 1

[a]: 2 patients screened at admission were readmitted during the control phase.

[b]: 6 patients stayed both in the interventional and control phase.

[c]: information available for 151 and 312 patients in the interventional and control period.

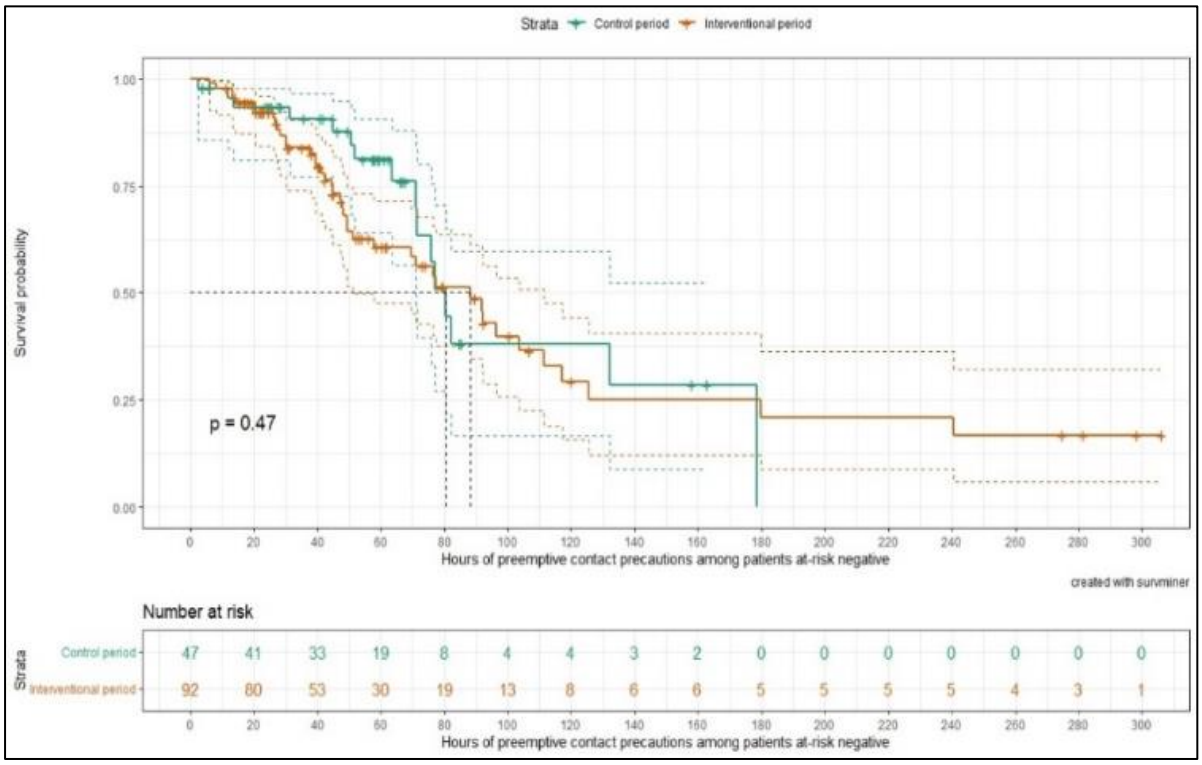
Colonization pressure was similar between interventional and control periods for both ESBL-PE and CPE, but not for CPO, which increased during the control period (Table 1). Median antibiotic consumption and hand hygiene compliance were also similar between both periods, though minor monthly variations occurred (Table 1, Suppl. Table 2). Adherence to weekly screening during both interventional and control phases, was 69.6% [95%CI 61.5-77.5] and 84.0% [95%CI 71.1-87.8], respectively. Lowest screening rates were observed in March 2020 at the peak of the first COVID-19 pandemic wave (Suppl. Figure 1). We performed 23 audits to assess implementation of CP. An agreement of 94.0% (146/156 observations) was observed between prescribed and implemented CP.

Among all screened patients, ESBL-PE prevalence was 16.1% (168/1'043), including 4.3% (45/1'043) of nEcESBL-PE and 1.3% (14/1'043) of CPO. Most of the nEcESBL-PE, and CPO were isolated in patients at high risk (respectively 16% and 5%; Suppl. Table 3). Among 1'117 samples, including 25 samples CPO-positive by LAMP, the diagnostic performance indicators were: 91.7% [95%CI 76.0-100.0] sensitivity, 98.7% [95%CI 98.1-99.4] specificity, 44.0% [95%CI 24.5-63.5] positive predictive value (PPV), and 99.9% [95%CI 99.7-100.0] negative predictive value (NPV). Among 27 samples nEcESBL-PE positive

by LAMP, performances were: 45.4% [95%CI 28.5-62.4] sensitivity, 98.8% [95%CI 98.1-99.5] specificity, 55.6% [95%CI 36.8-74.3] PPV, and 98.2% [95%CI 97.3-99.0] NPV. To note, specificity and NPV observed among CPO (98.7%, 100.0%) and ESBL-producing non-*E.coli* (96.0%, 92.0%) decreased among patients at a high risk screened at admission. Further analysis of discordant results unveiled that most of isolates negative by LAMP were positive once retested (Suppl. Appendix 3).

Of 92 and 47 patients screened at admission with unnecessary preemptive CP during the interventional and control period, we observed a median time from admission to CP discontinuation of 88.3 (95%CI 57.7-103.7) versus 80.5 (95%CI 71.5-132.1) hours (p=0.47, Figure 2). Time from admission to result notification was respectively 21.1 (95%CI 18.5-25.8) and 103.1 (95%CI 66.4-131.3) hours (p <0.001). Following univariate competing risk regression, the rapid screening strategy did not accelerate discontinuation of CP (sHR 1.4 [95%CI 0.8-2.6], p=0.2), with similar estimates using Cox regression. Results were unchanged after the exclusion of patients screened during weekends and laboratory holidays, as well as after the exclusion of patients at a high risk (Suppl. Appendix 4, Suppl. Figures 2 & 3).

Figure 2. Time (hours) spend under preemptive contact precautions by negative patients screened at admission with culture-based methods (control period) and LAMP assay (interventional period)



Among patients admitted during weekdays and screened at admission in the interventional and control period, 34 (37.0%) and 14 (29.8%) had CP discontinued in the ICU. Among them, CP discontinuation occurred 43.4 (IQR 27.0-92.0) and 67.4 (34.7-84.6) hours after admission (p=0.29, Table 2) during the interventional and control period, respectively, and results were notified 22.1 (IQR 12.3-55.2) and 61.9 (56.7-105.0) hours after admission (p<0.001). No apparent impact of the first pandemic wave was observed on pre-analytical and post-analytical TATs (data not shown).

Table 2. Laboratory turn-around times among patients at-risk with unnecessary contact precautions and actionable results (excluding patients at a high risk of carriage and patients screened during holidays)

	Interventional period (n=34)	**Control period** (n=14)	**P-value**
Pre-analytical TAT			
From admission to screening (h)	11.6 (IQR 2.0-21.8)	6.2 (IQR 3.2-33.3)	0.759
Analytical TAT			
From screening to arrival in the laboratory (h)	2.5 (IQR 1.5-11.3)	6.4 (IQR 2.3-19.0)	0.189
From receipt to result notification (h)[a]	2.6 (IQR 2.1-28.8)	40.4 (IQR 29.3-73.7)	<0.001
Post-Analytical TAT			
From result notification to CP discontinuation (h)[a,b]	24.0 (IQR 5.7-32.8)	17.4 (IQR 9.1-30.5)	0.56
Total TAT			
From admission to CP discontinuation (h)	43.4 (IQR 27.0-92.0)	67.4 (IQR 34.7-84.6)	0.29
From admission to result notification (h)[a]	22.1 (IQR 12.3-55.2)	61.9 (IQR 56.7-105.0)	<0.001

Footnote to table 2:

[a] Excluding 2 patients in the interventional period with missing date of results.

[b] Excluding 3 and 5 patients in the interventional and control period with CP discontinued before results notification.

CP were implemented for two patients newly CPO-positive by LAMP. However, they were false positive by culture, leading to unnecessary contact precautions. Considering the poor PPV of this test, the ICU physicians decided to stop using the LAMP assay on rectal swabs in June 2019.

Incidence densities of acquisition per 1'000 patient-days during the interventional and control period were respectively 2.48 [95%CI 1.55-3.75] and 2.92 [95%CI 1.46-5.22] for nEcESBL-PE; 0.34 [95%CI 0.07-1.00] and 2.12 [95%CI 0.92-4.18] for CPO; and 0.11 [95%CI 0.03-0.81] and 1.06 [95%CI 0.29-2.72] for CPE only. Incidence rate ratios for nEcESBL-PE and CPO acquisition were 0.80 [95%CI 0.36-1.75; p=0.57) and 0.23 [95%CI 0.03-1.76; p =0.16) after adjustment for colonization pressure and hand hygiene compliance.

Discussion

The findings of this interventional cohort study support three main conclusions: (1) the diagnostic accuracy of the LAMP assay performed directly on rectal swabs was suboptimal; (2) under real-life conditions, there was no benefit of this rapid diagnostic strategy in a low-endemicity setting, neither for discontinuing unnecessary CP among critically ill patients screened at admission, nor for implementing CP among newly positive patients; (3) many ICU patients screened at admission and placed under preemptive CP were negative, and most of them were discharged before discontinuation of CP.

The rapid screening strategy had methodological flaws. Although it demonstrated acceptable NPV for discarding intestinal carriage of nEcESBL-PE and CPO, it generated several false positive results as compared to cultures. The low endemicity and poor pre-test probability during universal weekly screening both impacted the observed PPV. Because of the human and economic cost of unnecessary CP, the ICU physicians decided to stop using the rapid screening strategy to implement CP early in the study. Moreover, the investigation of discordant results observed that when retesting isolates from previously LAMP-negative swabs, they are often identify as positive. This indicates

that the quality of rectal screening might have impacted analytical sensitivity.

Unfortunately, few samples were available to re-examine swabs positive by LAMP but negative by cultures. We hypothesized these discordant results might partly be explained by under-detection from cultures, in case of low bacterial load, non viable species on specimen, growth difficulty in selective media for non-Enterobacterales species (e.g. non-fermentative bacteria), enzyme mutants not expressing carbapenemases activity,(149) and for certain resistance mechanisms with low hydrolytic activity (OXA-48-like enzymes), which has also been observed in prior studies,(150,151) with unclear infection control relevance.(151)

Despite reduced analytical TAT, the overall duration of unnecessary CP among patients screened at admission was not significantly different between the intervention and control periods, even after exclusion of patients screened during holidays or at a high risk of carriage. Of note, only a fraction of eligible patients screened at admission had CP discontinuation before ICU discharge. This can be explained by the short length of ICU stay, and slightly higher pre- or post-analytical TATs during the interventional period, suggesting a role for external factors. (152)

We observed a non-statistically significant increase of CPO acquisition rates during the control period, but not regarding nEcESBL-PE. This increase was confounded by an outbreak of VIM-producing *P. aeruginosa* (n=21) from April 2018 to September 2020 related to an environmental reservoir, with a peak observed in August 2020.(153) However, similar conclusions were observed regarding acquisition rates after exclusion of non-fermentative bacteria. We are therefore confident to conclude that the rapid screening strategy did not change nEcESBL-PE and CPO acquisition rates.

Several experimental studies observed reliable concordance of the eazyplex® SuperBug CRE system when performed on CPO and nEcESBL-PE isolates when compared to cultures or PCR.(154–157) However, when directly performed on rectal swabs, Yamamoto et al. observed a PPV of 62% to detect carbapenem-resistant *Acinetobacter baumannii*,(130) which is close to our PPV. The sole study evaluating clinical relevance of LAMP when performed on rectal swabs and/or bronchial aspirates, observed a decreasing incidence of carbapenem-resistant *A. baumannii* infection from 35.2 to 20.9 per 1'000 patient days in a hyper-endemic ICU using weekly, admission, and discharge screening.(130) Another benefit of such rapid test has been suggested by a study using PCR to accelerate screening during outbreaks.(158) However, one should also consider current limitations to implement LAMP tests in routine screening, which include their cost, and the additional workload to simultaneously process cultures and LAMP tests.

This study is the first to evaluate clinical effectiveness of a rapid screening strategy based on LAMP tests to accelerate discontinuation or implementation of infection control measures. However, our study has several limitations. First, the study design did not allow a concurrent control group, which left room open for confounding events such as the SARS-CoV-2 pandemic and VIM-producing *P. aeruginosa* outbreak, with a possible influence on surveillance and implementation of infection control measures. Second, the microbiological laboratory of our institution automated its plating and incubation processes in March 2019, decreasing TAT of cultures.(146) Comparison of LAMP with a competing, improved control could potentially under-estimate its true benefits. However, the major problem remained unchanged, which was the short length of ICU stay as compared to the time for CP discontinuation. Third, results might not be generalizable to hyper-endemic settings.

Conclusions

In its current form, a rapid rectal screening strategy based on LAMP assays has neither a clear benefit to discontinue unnecessary CP among patients screened at admission, nor an added value to accelerate the implementation of CP among newly positive patients in a low-endemic setting. This study suggests the requirement for further adjustments, including IT-based automatic reporting of molecular resistance information combined with IPC stewardship to ensure fast and reliable use of results, and further control to improve the quality of rectal swabs. Further research could investigate benefits from LAMP to fasten unit-wide screening for outbreak control, or IPC measures in hyper-endemic settings.

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Supplementary Appendix

Supplementary Appendix 1. Adherence to screening and contact precautions

Weekly surveillance screenings are often not performed for patients already screened recently (admission screening), and could also be delayed by several days. Therefore, estimating true adherence to weekly surveillance screening should account for prior and delayed screenings. Instead of measuring adherence of screening only on Monday, which would underestimate the true proportion of patients screened, we opted to measure the screening coverage of all ICU patients hospitalized on Monday from 05 am to 08 am (candidates for weekly screening). Among this population, the screening coverage considered those with a screening performed from the prior Tuesday to the next Wednesday. This indicator helps to answer whether carriage status was investigated among patients present at the time of weekly universal screening. During the interventional phase, we investigated missing screenings, and distinguished screening performed elsewhere, performed in ICU but not included in the study, or not performed. Adherence to prescribed CP was assessed by 4 audits spanning the interventional and control period.

Supplementary Appendix 2. Risk factors for patients screened at admission

Of 231 patients with a targeted screening at admission, we distinguished 58 (25%) patients at a high risk requiring sequential screening, and 173 (75%) patients at risk (Table 1). Most frequent high risk exposures included 27 (46%) previously known carriers, 16 (28%)

direct transfer from or recent hospitalization in ICUs abroad, and 15 (26%) prior hospitalization in endemic countries. Most frequent exposures defining patients at-risk included 92 (53%) hospital transfer, 18 (10%) prior hospitalization in Swiss or French hospital, 32 (18%) other reasons, and 22 (13%) unknown reason. Among all patients screened upon admission, 185 (80%) had CP implemented at admission. Among them¸46 (25%) had an indication to keep CP, including other MDRO carriage.

Supplementary Appendix 3. Investigation of results with discordant LAMP and culture results

LAMP positive and culture negative.

Because of quality concerns, only four from 23 samples with positive LAMP results and negative culture results were further investigated. 14 of 23 results were discordant for CPO results (6 KPC, 3 OXA-181, 3 NDM, 1 OXA-48, 1 KPC & NDM), and 12 of 23 were discordant for ESBL results (9 CTX-M-1, 3 CTX-M-9). Two samples with discordant ESBL results, and two samples with both discordant ESBL and CPO results were retested using PCR, which did not confirm initial results, and detected TEM genes.

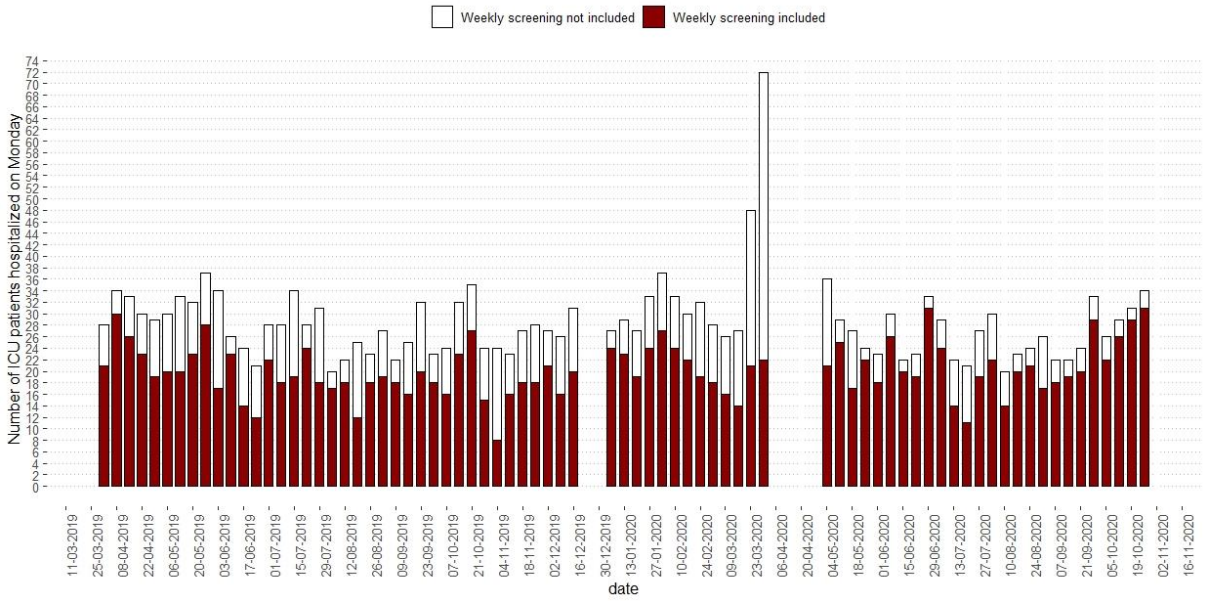
LAMP negative and culture positive.

27 of 31 isolates negative for ESBL by LAMP, including 21 *E.coli*, 9 *Klebsiella,* 1 *Citrobacter*, 1 *Enterobacter*, and 1 *Pseudomonas* species*,* were further investigated. Sixteen isolates were LAMP positive (11 CTX-M-1, 4 CTX-M-9, 1 for both CTX-M-1 & CTX-M-9), 4 additional isolates were confirmed as non ESBL-PE by disk diffusion methods, and among the 6 isolates tested by PCR, 2 were positive for TEM and SHV, 2 were positive for SHV, and 2 were negative.

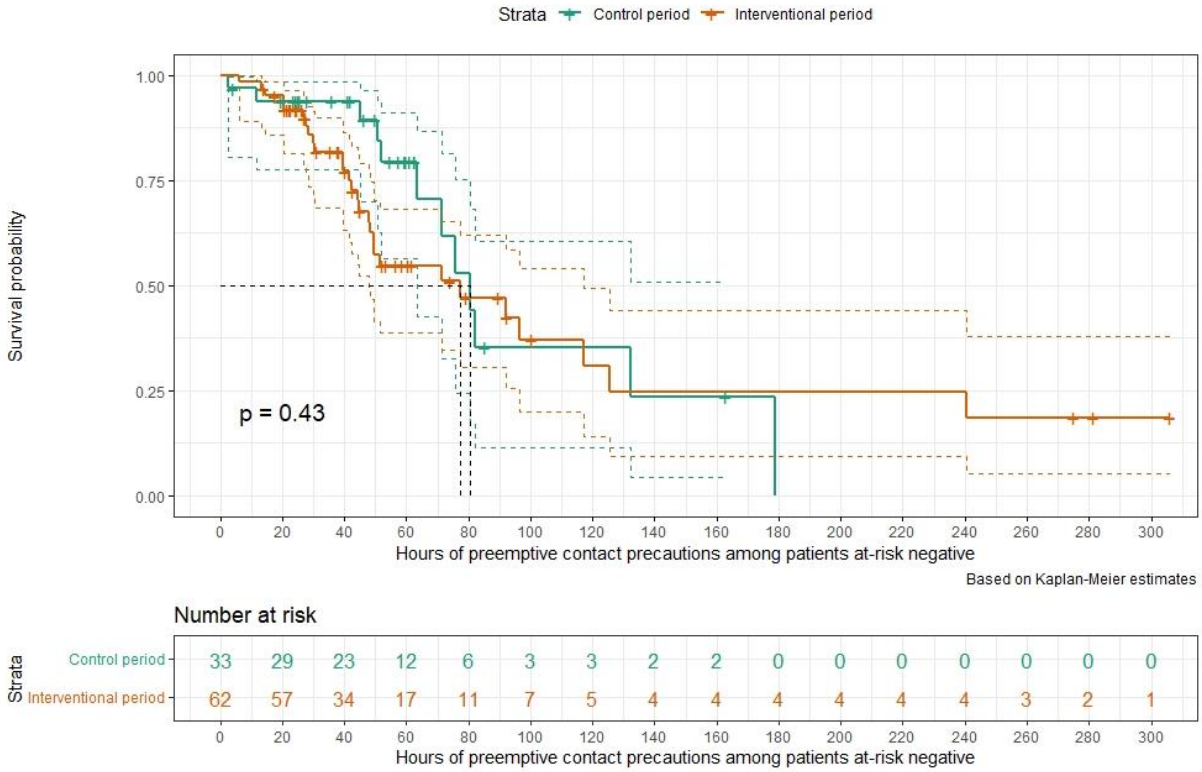
Supplementary Appendix 4. Time to discontinue contact precautions among patients at-risk screened upon admission, after exclusion of patients at a high risk, and patients screened during weekends and laboratory holidays.

Results were unchanged after the exclusion of patients screened during weekends and laboratory holidays, (n=95, 77.4 [95%CI 48.1-117.2] and 80.5 [95%CI 63.5-132.1] hours for interventional and control period, p=0.43), as well as after the exclusion of patients at a high risk (n=96, 51.3 [95%CI 44.8-88.3] and 75.9 [95%CI 71.2-82.1] hours for interventional and control period, p=0.06, Suppl. Figures 2 & 3).

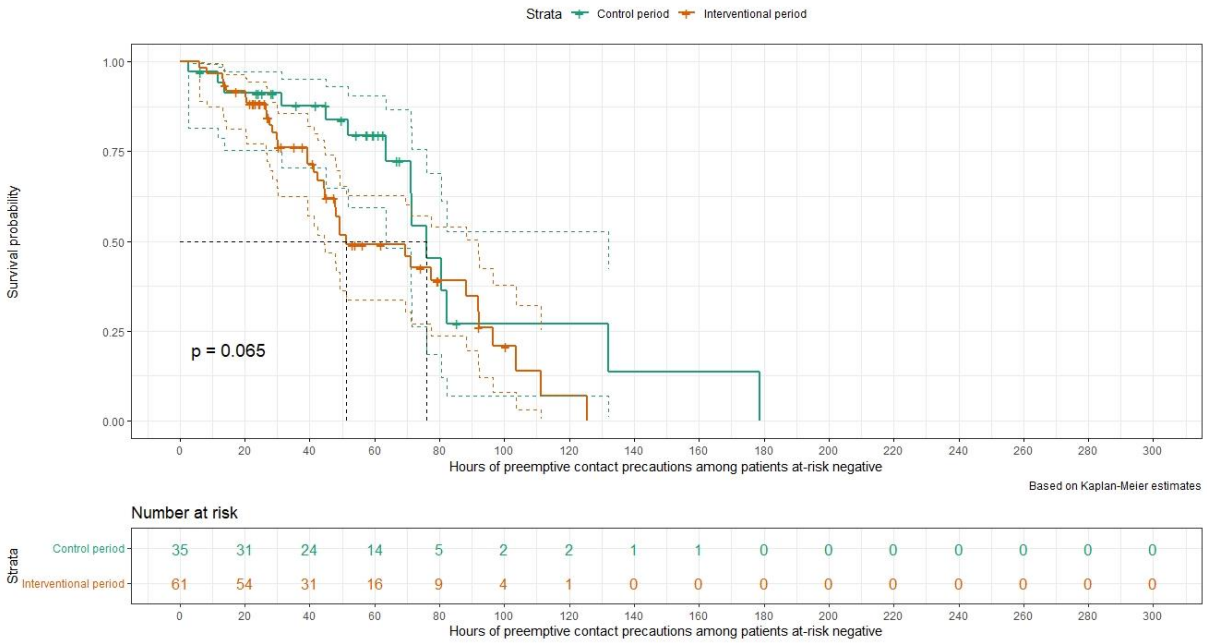
Suppl. Figure 1. Weekly screening coverage for patients hospitalized on Monday mornings from 5am to 8am.



Suppl. Figure 2. Time (hours) spend under preemptive contact precautions by negative patients screened at admission with culture-based methods (control period) and LAMP assay (interventional period) excluding patients screened from Friday to Sunday and during laboratory holidays



Suppl. Figure 3. Time (hours) spend under preemptive contact precautions by negative patients screened at admission with culture-based methods (control period) and LAMP assay (interventional period) excluding patients at a high risk and patients screened during laboratory holidays



Suppl. Table 1. Exposures considered for admission screening

Indication for ESBL-PE and CPO screening at admission of patients at-risk
Hospitalized & overnight stay in Switzerland
Prior travel in endemic countries
Dialysis treatment
Indication for ESBL-PE and CPO screening at admission of high risk patients
Known ESBL-PE and CPE carrier
Hospitalized & overnight stay abroad

Suppl. Table 2. Monthly hand hygiene compliance among healthcare workers in ICU

Date	Actions	Opportunities	Compliance	95% CI
2019-05-01	31	55	56,4	[95%CI 43.3-69.5]
2019-06-01	31	60	51,67	[95%CI 39-64.3]
2019-08-01	17	26	65,4	[95%CI 47.1-83.7]
2019-09-01	47	76	61,8	[95%CI 50.9-72.8]
2019-10-01	53	79	67,1	[95%CI 56.7-77.5]
2019-11-01	30	47	63,8	[95%CI 50.1-77.6]
2019-12-01	25	46	54,3	[95%CI 40-68.7]
2020-01-01	18	28	64,3	[95%CI 46.5-82]
2020-02-01	27	47	57,4	[95%CI 43.3-71.6]
2020-03-01	4	10	40.0	[95%CI 9.6-70.4]
2020-04-01	15	28	53,6	[95%CI 35.1-72]
2020-05-01	17	42	40,5	[95%CI 25.6-55.3]
2020-06-01	23	31	74,2	[95%CI 58.8-89.6]
2020-07-01	63	110	57,3	[95%CI 48-66.5]
2020-08-01	78	117	66,7	[95%CI 58.1-75.2]
2020-09-01	39	60	65	[95%CI 52.9-77.1]

Suppl. Table 3. Prevalence of patients carrying EC-ESBL, nEC-ESBLPE, CPE, and CPO among 3 sub-cohorts of ICU patients.

	Intervention period			**Control period**		
	Patients at-risk at admission (n=117) [a]	Patients at a high risk at admission (n=30)[b]	Patients screened weekly (n=589)[c]	Patients at-risk at admission (n=57) [a]	Patients at a high risk at admission (n=29)[b]	Patients screened weekly (n=313)[c]
Patient carrying ESBL-producing *E.coli*	15 (12.8%)	8 (26.7%)[e]	81 (13.8%)	7 (12.3%)	9 (31.0%)	36 (11.5%)
Patient carrying nECESBL-PE	5 (4.3%)	5 (16.7%) [e]	19 (3.2%)	0 (0.0%)	8 (27.6%)	16 (5.1%)
Patient carrying CPE	0 (0.0%)	1 (3.3%)	3 (0.5%)	0 (0.0%)	3 (10.3%)	3 (1.0%)
Patient carrying CPO	0 (0.0%)	1 (3.3%)	4 (0.7%)	0 (0.0%)	3 (10.3%)	8 (2.6%)

Footnote

[a]: 1 patients was included both in the interventional and control phase.

[b]: 1 patients were included both in the interventional and control phase.

[c]: 6 patients were included both in the interventional and control phase, patients at-risk were included if screened weekly

CHAPTER FOUR

General discussion

Introduction

This thesis aimed to elucidate the transmission dynamics and temporal trends of antibiotic-resistant Gram-negative bacteria, more specifically of ESBL-producing *E. coli* and *K. pneumoniae* in understudied settings, such as LTCFs and households. First, we aimed to assess the proportion of co-carriage and transmission of ESBL-producing *E.coli* and *K.pneumoniae* among household members, the temporal trends in the prevalence of ESBL-EC clones in a LTCF, and the epidemic potential of emerging ESBL-EC subclones in a LTCF. Second, this thesis attempted to improve active surveillance strategies by measuring existing gaps and barriers in Switzerland, and by evaluating innovative screening methods in accelerating infection control measures targeting MDR-GNB. Specifically, we aimed to evaluate current MDRO admission screening practices in Swiss hospitals and barriers impeding their implementation, and to compare traditional phenotypic methods against rapid screening strategies to accelerate the discontinuation of unnecessary preemptive CP for negative patients screened at admission, and the implementation of infection control measures for newly identified carriers.

Summary of results

The **first chapter** aimed to improve our comprehension of ESBL-PE dynamics and to monitor epidemiologically important multidrug resistant clones with high transmissibility in understudied settings such as households and LTCFs. In the **first part** of this chapter, based on an investigation in a university-affiliated LTCF, we observed a high prevalence of ESBL-EC and its clone ST131 (10.5%, and among them 58.0%). ESBL-EC prevalence increased from 2010 to 2020, while its ST131H30 subclone decreased. However, we fortuitously detected the clonal expansion of an atypical subclone ST131H89 from 2018 driven by multiple silent outbreaks. Despite a short follow-up period, the absence

of rebound effect following the discontinuation of contact precautions for ESBL-EC in 2019 supported the most recent guidelines for ESBL-PE control. The prevalence of ST131 clones among all ESBL-EC in our LTCF was comparable to LTCF in other countries such as The Netherlands or Spain (56-70%) (1,2). Though clonal fluctuation with displacement of existing subclones is a known phenomenon in ESBL-EC epidemiology, (3) the spread of the specific ST131H89 subclone is unusual and has not been reported yet. The effectiveness of standard precautions alone to control ESBL-EC has already been observed in several other studies, performed either in acute-care hospitals or in LTCF. (4–6) The **second part** of this chapter focused on ESBL-PE transmission dynamics in household settings. In a systematic review, we aimed to quantify the prevalence of ESBL-PE co-carriage among families. In 13 studies, 8% to 37% of 863 household members of ESBL-PE positive index cases were also colonized by an ESBL-PE. More precisely, 12% (95%CI: 8-16%) among these household members were colonized by a clonally-related ESBL-PE strain, with higher proportions for index cases carrying *K. pneumoniae* as compared to *E.coli* (20-25% versus 10-20%). In a subset of relevant studies, acquisition rates of clonally-related ESBL-PE among 180 initially ESBL-PE free household members of a previously identified carrier ranged between 1.56 - 2.03 events per 1000 person-weeks of follow-up. In summary, this review highlighted the role of families and households as ESBL-PE amplification platform, supported by pre-existing evidence. To note, multiple sources of ESBL-PE introduction with shared exposure among household members could contribute to the high prevalence observed in families and the polyclonal ESBL-PE picture. This systematic review also highlighted an important heterogeneity among studies and methodological gaps. Index patients presenting asymptomatic carriage and infection from endemic or epidemic settings were mixed, and external confounding by antibiotic, healthcare, travel, or food exposure was frequent. Different designs such as cross-sectional and cohort

studies, as well as selection of index cases and household contacts further impeded comparison between studies. Robust detection methods with sequencing of multiple isolates per morphotype were recommended for future research. **The third part** of the first chapter was a direct continuation from the prior systematic review, with a multicentric prospective cohort of ESBL-EC and ESBL-KP colonized index cases and their household members, using whole genome sequencing to determine acquisition and transmission events, and adjusting with relevant exposures such as comorbidities, antibiotic, proton pump inhibitor therapy, hospital, travel, and food exposure. Among 71 index cases and 102 household contacts, the incidence of ESBL-PE transmission among households was 1.18 per 100 participant-weeks at risk, with higher rates for ESBL-KP against ESBL-EC (1.16 versus 0.93 per 100 participant-weeks at risk). Interestingly, most ESBL-PE acquisitions occurred during the first 2 months (1st week: 41%; 2nd-8th week: 29%). Providing assistance for urinary and faecal excretion to the index case increased the risk of ESBL-PE transmission among household members (adjusted prevalence ratio, 4.3; 95%CI 1.3-14.1). This study observed higher co-carriage proportions compared to those described in the former systematic review (34% with 1.9 events per 100 weeks at risk versus 12%, with 0.16 to 0.20 events/100 participant-weeks). This significant difference could be explained by a reduced risk of detection bias in the later cohort study, using enrichment broths, and selecting multiple colonies per samples. Furthermore, the shorter follow-up duration in the cohort study (4 versus 12 months) probably contributed to capture more transmission events.

The **second chapter** evaluated the implementation status of screening strategies and infection control measures among multiple hospitals at the national level. It also aimed to assess the effectiveness of certain screening strategies, including universal regular screening and a rapid

screening strategy included as part of the universal regular and targeted admission screening. In **the first part**, a nation-wide survey among 139 Swiss healthcare institutions was conducted, achieving a good response rate and an adequate coverage of the Swiss health system (covering 49.5% of all 281 recorded healthcare facilities in Switzerland). (7) The difference mostly resulted from excluded specialized clinics (psychiatric institutions, palliative care, pain therapy centers), rehabilitation centers and LTCFs. This survey observed that MDRO admission screening was implemented in 83% of institutions, with striking differences between private and public institutions (28% versus 9% did not implement admission screening), and mostly including CPE, ESBL-PE and MRSA. However, surveillance gaps at admission were identified for VRE (44 % of institutions), multi-resistant *A. baumanii* (41 %) and *P. aeruginosa* (37 %). Interestingly, admission screening practices for VRE were mostly deficient in Easter Switzerland, and coincided with large multi-hospital VRE outbreaks, which required revision of national guidelines for VRE control. (8,9) This survey also identified heterogeneity among risk factors and body sites used in surveillance. To note, the difficulty to identify high-risk patients was mentioned as a barrier in 44% of participants. These findings highlighted the need for harmonized and feasible screening strategies targeting resistant Gram-negative bacteria among Swiss healthcare institutions. The difference with the lower screening rates previously reported in Germany or in The Netherlands is probably due to an increased awareness of institutions over time. (10,11)

In the **second part**, we detailed the early control of a NDM-producing *E.coli* institutional outbreak following fortuitous detection of secondary cases by regular universal screening in the ICU. This small outbreak investigation highlights the added value of universal regular screening to facilitate early implementation of infection control measures.

In the **third part**, we performed an interventional quasi-experimental study comparing a rapid genotypic test with standard phenotypic

cultures, in order to accelerate surveillance and subsequent infection control measures. We observed a suboptimal diagnostic accuracy of the rapid LAMP assay for ESBL-PE and CPE when directly performed on rectal swabs. The poor positive predictive values reproduced the estimates already published from multiplex PCR and LAMP directly performed on rectal swabs. (12–14) Importantly, most of ICU patients screened at admission and under unnecessary contact precautions were discharged before discontinuation of contact precautions. Our study observed that under real-life conditions, and without proper diagnostic stewardship and further adjustments (quality control of rectal swabs sampled in surveillance screening), there was no benefit of LAMP in a low-endemicity setting, neither for discontinuing unnecessary CP among critically ill patients screened at admission, nor for implementing CP among newly positive patients. However, these parameters heavily depend on epidemiological settings and local prevalence of pathogens of interests.

Strengths and limitations

First, this thesis evaluated the dynamics of ESBL-PE in understudied settings, such as households and LTCFs. These settings constitute an important reservoir considering the specific vulnerability of LTCF and the community dissemination of ESBL-PE. Traditional challenges impacting findings from household-based prospective cohorts were highlighted in our review and accounted for in our later prospective cohort. **Secondly,** we monitored several atypical clones at risk, including NDM-producing *E.coli* ST354 in acute care facilities and ESBL-producing *E.coli* ST131H89 in LTCFs, advocating to further monitor the molecular epidemiology of certain clones among LTCF using repeated cross-sectional surveys and among institutions using regular universal screening in high-risk units (i.e. ICUs). **Third**, our repeated prevalence surveys in LTCFs benefited from a large sample size and long-term surveillance data. **Fourth**, the

originality of our review was a strength, opening a path for future prospective cohort studies among household settings. **Fifth,** the subsequent, original cohort study used a robust methodology and detection methods by using whole-genome sequencing in a multicentric population to ascertain ESBL-PE transmission rates in households. **Sixth,** our nation-wide survey achieved an excellent response rate covering a large proportion of public and private Swiss healthcare institutions and combining results with geo-spatial information. Such information could have major impact when interpreted simultaneously with epidemiological data, such as the number and size of MDRO clusters, or prevalence of healthcare-associated MDROs. **Seventh,** our quasi-experimental study was original in demonstrating lack of effectiveness of a rapid screening test to inform infection control measures, and benefited from robust exhaustive admission and weekly screening strategies.

However, the scientific work leading to this thesis has also several limitations. **First,** the COVID-19 pandemic (2020-2022) impacted not only the productivity and consistency of the research produced in this thesis, but also interfered with the design, conduct and analysis of several studies. Laboratories, researchers, and clinicians were either forced to delay or to reallocate their time and funds for COVID-19 related projects. Many COVID-19 studies were conducted by our group in order to guide infection control policies both in hospitals and the community, but remained outside the scope of this thesis. Studies occurring during the pandemic period were often influenced by residual confounding effect and bias, including sampling bias (reallocation of testing resources), modified application and adherence to screening strategy, but also to standard and contact precautions. For example, the interventional quasi-experimental study, already prone to confounding events by using a historical control, was probably influenced by decreased screening rates, by suboptimal infection control measures (temporary universal gloving), and by

modified opportunities at risk for cross-transmission (increased self-awareness). Decreased screening rates possibly under-detected potential acquisitions or transmissions occurring in ICUs. A **second limitation** was the late detection of a nosocomial VIM-producing *P. aeruginosa* outbreak occurring in the Geneva ICU between 2018 and 2020. As previously described, the control of this outbreak included temporary ICU closure of certain areas, with enhanced patient screening (biweekly screening) and environmental control. (15) The co-occurrence of the COVID-19 pandemic and this *P.aeruginosa* outbreak and their unmeasured effect on our findings illustrates the vulnerability of interventional IPC studies to external confounding and time effects. The **third** limitation is related to the challenges of conducting prospective studies in household settings, and include potential selection bias when including household members and strains to be sequenced. As a **fourth** limitation, the heterogeneity of surveillance and infection control measures implemented globally, and the influence of epidemiological context and resources on their implementation and effectiveness impair the generalizability of our findings. A **fifth** limitation related to the quasi-experimental study in the ICU is related to the absence of gold standard when evaluating the LAMP assay, with the uncertain clinical relevance of discordant LAMP positive results. Such discordances might partly be explained by under-detection from cultures, in case of low bacterial load, non-viable species on specimen, growth difficulty in selective media for non-Enterobacterales species (e.g. non-fermentative bacteria), enzyme mutants not expressing carbapenemase activity, (16) and for certain resistance mechanisms with low hydrolytic activity (OXA-48-like enzymes), which has also been observed in prior studies, (17,18) with unclear infection control relevance. (17) Interpretation of molecular resistance information to guide IPC measures might be hindered by these discrepancies. An additional limitation of using molecular tests, not addressed in this manuscript, is the changing landscape of resistance genes. In summary,

emergent resistance genes (false negative) or mutant resistance genes (false positive) might or might not be detected by molecular tests. **Sixth**, this thesis highlighted the presence of potential delays when identifying target populations for screening upon admission. Effectively, certain exposures are only detected after thorough discussion. Difficulties in identifying patients at risk can also result in a potential information bias with patients not screened. However, the most relevant exposures are systematically informed at admission, such as direct transfer from a healthcare institution or previously known carriage. Additional delays result from the interpretation of complex results, often requiring an IPC consultation. Thus, when evaluating the effectiveness of a rapid test to accelerate infection control measures, strategies to accelerate identification of patients at risk, and interpretation of molecular resistance information should be included as part of a holistic screening strategy, with a defined diagnostic stewardship program. **Seventh**, ethical constraints obstructed the implementation of a robust design evaluating ecological benefits from routine interventions. A cluster-controlled trial was initially planned to assess the effectiveness of LAMP assays. As our approach was based on an already pre-existing MDRO surveillance and control strategy targeting critically ill patients, a waiver of informed consent according to the Art. 34 LRH was deemed necessary. Unfortunately, despite multiple appeals and well-thought justifications, the waiver of informed consent was not granted by our local IRB. Thus, in agreement with the president of the ethical committee, the study design was modified for a quasi-experimental study without cross-over that did not require informed consent. The discussion with our ethical committee and further modifications in our protocol further delayed the ICU study start until April 2019.

Connection with existing policies, practices, and instruments

In the **first part** of the **first chapter,** our repeated surveys of ESBL-EC prevalence in LTCF observed no rebound effect after discontinuation of contact precautions, and clonal fluctuation with multiple silent monoclonal outbreaks of ESBL-EC. These findings are supporting current guidance recommending discontinuation of contract precautions for ESBL-EC in healthcare settings. The emergence of closely related clones, sometimes associated with oxacillinases in neighbouring countries (19) warrants a close monitoring of ESBL-EC subclones, especially regarding the current under-detection of the OXA-48 carbapenemase and the absence of genotypic confirmation of CPE in certain countries. The silent outbreaks in LTCF highlights their vulnerability regarding MDRO spread and advocates for surveillance in this setting, such as repeated prevalence surveys.

In summary, this thesis suggests for existing surveillance networks to:

- Monitor carefully the emergence of certain ESBL-EC subclones, particularly in high-risk settings such as LTCFs using simple and low-cost designs such as repeated cross-sectional surveys.

In **the second and third parts**, our systematic review and its following prospective cohort study observed a significant rate of acquisition and transmission among household members of an index patient colonized by ESBL-PE. Particularly, index patients requiring assistance for urinary and faecal excretion were at increased risk to transmit ESBL-EC or ESBL-KP to caregivers. This specific exposure is currently not included in defined risk profiles targeting candidates for admission screening. However, the feasibility to identify this subpopulation at admission and the positive

predictive value of this exposure among screened patients still need to be determined. Furthermore, information sheets could be provided to household members to raise awareness of the increased risk of cross-transmission and to limit community spread.

In summary, this thesis suggests for institutions to:

- Consider household contacts of ESBL-PE index cases as risk profiles defining candidates for targeted admission screening
- Inform household members carrying for ESBL-PE or CPE positive patients with impaired autonomy about their increased risk of cross-transmission, with recommendations for good hygienic practices in order to limit household spread.

In the **first part** of the **second chapter**, our national survey on screening practices at admission participated in identifying current gaps in the institutional and national MDRO surveillance system, especially concerning certain neglected pathogens and risk factors. This survey also observed a significant heterogeneity in current practices, with identification of major facilitators and barriers to implement correct practices. These findings advocated for harmonized national guidance to better control importation events and inter-facility transfers. These findings have been communicated to SwissNoso and particularly the StaR committee and might serve as an evidence base to strengthen national guidelines. They also have been mentioned and could support the current update of ESCMID guidelines to control nosocomial ESBL and CPE spread. Homogenized screening policies are particularly warranted to avoid importation events, regarding the recent Swiss VRE outbreaks in multiple facilities, the emergence of OXA carbapenemases, and the presence of community clones (E.coli ST131 H41) harbouring carbapenemases.

In summary, this thesis suggests for policy makers to:

- Harmonize risk profiles used to target candidates for admission screening by easily accessible standards of evidence-based risk factors for MDRO colonization and infection. Such risk profiles might facilitate identification of patients at-risk in clinical routine.
- Potentially include in these standards an evidence-based list of body sites to be screened for each pathogen considered for admission screening.
- Potentially consider repeated surveys of screening practices among healthcare institutions and integration with epidemiological surveillance to prevent large inter-institutions outbreaks.

In the **second part,** the failure of a rapid genotypic screening strategy in accelerating infection control and the challenges observed in this interventional study might advocate for abandoning the use of rapid genotypic assays without a proper diagnostic stewardship programme facilitating identification of target populations, result notifications and interpretations. Rapid phenotypic tests might yield an advantage to identify species and facilitate interpretation of results with key relevant information (viability, clinical and IPC relevance). We observed no individual nor ecological harm by using this rapid test in the surveillance screening strategy. This risk-benefit balance might be used as an example to advocate more flexible use of waivers of informed consent in Swiss laws concerning future prevention trials in Switzerland, and also to highlight the importance of experts to ascertain on a case-by-case basis the adequate balance of risks against benefits.

In summary, this thesis suggests for healthcare institutions to:

- When implementing a screening strategy, include a diagnostic stewardship programme to accelerate the identification of target subpopulations, result notifications and interpretations.

In summary, this thesis suggests for ethical committees to:

- Use this example to advocate for more flexible use of waivers of informed consent in future trials assessing ecological benefits of surveillance or infection control practices in Switzerland.

Perspectives for future research

The findings from this thesis also raised additional scientific questions which could serve as a basis for future research activities.

The emergence of ESBL-EC ST131H89 in our LTCF, scarcely described in the literature by studies using globally-sourced genomes, (20) has never demonstrated monoclonal clustering in community or in healthcare settings, except from a recently published survey performed in 2019 among 16 Swiss LTCF, (21) from Swiss household members of our prospective cohort study, and from environmental samples isolated from a Swiss river. (22,23) An international research collaboration has recently been created to compare these strains in a multicentric study evaluating the regional spread of an atypical ESBL-EC subclone among different human and environmental reservoirs from Western Switzerland. Of note, a close *E.coli* subclone (ST131H41) has recently been observed to harbor an oxacillinase in neighbouring Germany. (19) This highlights the need for monitoring this atypical subclone on a larger scale.

The observational cohort of household members offered a robust overview of transmission dynamics of major ESBL-PE in the first months after discharge of an index patient, using sequencing information. Future research could complement missing epidemiological information to ESBL-PE transmission dynamics in the community. In particular, we miss robust estimates of the duration of colonization. This could be achieved by prospective cohort studies using a predefined long-term follow-up, robust screening strategy, and whole-genome sequencing to avoid potential attrition bias, selection bias, and detection bias. Previously negative index patients could be recruited following an outbreak or from units with high sampling rates to avoid potential lead time bias. Such study could serve to identify of ESBL-PE or CPE carriers with intermittent or long term carriage, and could ultimately inform surveillance practices in hospitals

and future mathematical modelling incl. agent-based models. In particular, this information could be used to strengthen the current indications and protocols for sequential screening practices among known carriers.

Highly heterogeneous IPC policies were observed at a national level between healthcare institutions, with several important gaps in surveillance and infection control practices. A follow-up survey could be informative, especially few years after the diffusion of nation-wide standards. If follow-up surveys are scheduled, the findings could be associated with the analysis of secular trends in nosocomial MDRO prevalence and incidence.

Using rapid genotypic tests without a proper diagnostic stewardship programme was not sufficient to accelerate infection control measures and had poor diagnostic value to detect ESBL-PE and CPE in a low endemic setting. However, the negative predictive value was adequate, and future studies could assess the effectiveness of this test to accelerate screening of close contacts for outbreak control, or to accelerate the cohorting of MDRO carriers in high-endemicity settings. However, the positive predictive value would remain a problem to identify carriers, and would probably require a two-tiers screening strategy with a more specific test. Also, methods to facilitate the identification of target populations presenting risk profiles at admission, result notification, and interpretation of molecular information could be developed to further accelerate surveillance and subsequent infection control measures. Finally, further research assessing effectiveness of rapid phenotypic methods to accelerate surveillance and improve infection control measures would probably yield interesting results.

Conclusions

This thesis contributed to the knowledge base about adequate monitoring of multidrug resistant clones and evaluation of transmission dynamics in understudied settings, such as LTCFs and household settings. We generated several original key findings. First, clonal fluctuation and silent outbreaks of ESBL-EC among LTCFs advocate for careful monitoring of emerging multi-resistant clones in similar settings. Second, we determined the role of households as ESBL-PE amplification platforms, especially for household members with impaired autonomy, with increased co-carriage proportions, either by significant acquisition and transmission rates, or potentially by sharing relevant exposures. This could inform future screening policies, IPC interventions to control community spread, or mathematical modelling. Third, heterogeneity and gaps were observed in admission screening policies among Swiss healthcare institutions. These findings could serve to inform future policy guidance, improving reallocation of existing financial and human resources by focusing on the right pathogens and exposure risks, while improving control of importation events and inter-facility transfers. Fourth, despite current controversies around universal MDRO surveillance to control MDR-GNB, this thesis highlighted an important side-benefit of such screening of critically ill patients to accelerate detection and early management of institutional outbreaks outside the ICU. Fifth, implementing a LAMP-based rapid genotypic test without proper diagnostic stewardship programme is doomed to fail in accelerating infection control measures. This lesson served as an additional evidence against routine use of molecular tests directly performed on rectal screening specimens in low-endemicity settings. Otherwise, diagnostic stewardship programme facilitating identification of target populations for admission screening, result notification and interpretation of molecular resistance information should be developed and included in institutional screening strategies.

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