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Scientific Research

Analysis of *Enterobacteriaceae* Producing Broad-Spectrum Beta-Lactamases in the Intensive Care Unit Setting*

Vendula Husičková[1#], Miroslava Htoutou-Sedláková[2], Ivanka Matoušková[3], Magdaléna Chromá[1], Milan Kolář[1]

[1]Department of Microbiology, Faculty of Medicine and Dentistry, Palacký University Olomouc, Olomouc, Czech Republic
[2]University Hospital Olomouc, Olomouc, Czech Republic
[3]Department of Preventive Medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc, Olomouc, Czech Republic
Email: [#]husickova.v@seznam.cz

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ABSTRACT

Strains of the *Enterobacteriaceae* family producing ESBL and AmpC broad-spectrum beta-lactamases that may survive in the hospital setting potentially cause infection in hospitalized patients due to contaminated objects or health care workers' hands. Over a period of two months (November-December 2010), a single epidemiological study of microbial contamination of air, surfaces and health care workers (swabs from both nostrils and the right hand without a glove) was carried out at two intensive care units of the University Hospital Olomouc, Czech Republic. The bacteria were identified using standard microbiological methods. Phenotypic detection of ESBL and AmpC enzymes and basic genetic analysis of ESBL- and AmpC-positive isolates was performed. The same approach was used to identify and analyze bacteria isolated from clinical samples of patients hospitalized at the above departments over the study period. From a total of 140 environmental samples collected over the study period, 21 isolates of the *Enterobacteriaceae* family were identified, with ESBL and AmpC production being detected in 4 and 7 isolates, respectively. Among patients' clinical samples, 10 ESBL- and 6 AmpC-positive isolates were detected. No similarity was found between environmental isolates and strains isolated from patients.

Keywords: *Enterobacteriaceae*; Broad-Spectrum Beta-Lactamases; Intensive Care Setting

1. Introduction

Members of the *Enterobacteriaceae* family are feared nosocomial pathogens, especially in high-risk departments such as intensive care, neonatal and burn units. The risk stems mainly from an increasing prevalence of ESBL- and AmpC-positive strains and the associated higher risk of failure of antibiotic therapy [1,2]. The growing number of *Enterobacteriaceae* spp. was also confirmed by data from the University Hospital Olomouc (UHO), Czech Republic. For instance, the percentage of *Klebsiella pneumoniae* isolates resistant to ceftazidime rose from 16% in 2005 to 40% in 2010 (unpublished data). The main mechanism of this resistance is production of broad-spectrum beta-lactamases, in particular ESBLs, that inhibit the effect of cephalosporin and penicillin antibiotics. Not uncommonly, isolates producing these enzymes are also resistant to other groups of antimicrobial agents such as fluoroquinolones, aminoglycosides, tetracyclines and co-trimoxazole [3,4]. In the UHO, fluroquinolone resistance of *Klebsiella pneumoniae* isolates increased from 12% in 2005 to 42% in 2010 (unpublished data). Besides *Klebsiella pneumoniae* strains, the common broad-spectrum beta-lactamase producers include *Escherichia coli* isolates and, less frequently, *Enterobacter cloacae*, *Serratia marcescens*, *Citrobacter* spp., *Proteus* spp. and others [5,6].

The prevalence of ESBL-positive strains also rises in "carrier" *Enterobacteriaceae* in the human gastrointestinal tract (GIT). Patients in the UHO were found to have 3% prevalence of ESBL-positive *Enterobacteriaceae* in the GIT in 2007 [7]. Carriage of these resistant bacteria is of particular importance to high-risk patients since they may cause endogenous nosocomial infections and serve as a reservoir for their further spread, including transferable genetic elements.

The potential sources of infection are patients and health care workers. However, suitable vehicles from the hospital environment may also play a role, in particular all wet areas where bacteria survive, e.g. sinks and drains. Most frequently, hand-touch sites are contaminated with Gram-negative bacteria, such as patient charts, tables,

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#Corresponding author.

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telephones and push plates [8]. There is clearly a dynamic relationship between the staff, patients and hospital environment, with bacterial strains being constantly transmitted [9].

With respect to the increasing bacterial resistance to antibiotics, measures have been introduced to lower the risk of nosocomial infections. These comprise antibiotic policy in hospitals including adequate application of antibiotics, proper hygienic disinfection of hands and the environment, isolation of the affected persons, search for contacts and, last but not least, continuous monitoring of resistant strains [10].

This prospective study aimed at determining the prevalence of *Enterobacteriaceae* producing ESBL and AmpC enzymes in two UHO intensive care units (ICUs) and performing their molecular biology analysis.

2. Material and Methods

Over a period of two months (November-December 2010), a single epidemiological study of microbial contamination of air, surfaces and health care workers (swabs from both nostrils and the right hand without a glove) was carried out in the UHO. The settings were the inpatient ward of the Department of Anesthesiology and Intensive Care Medicine (AIC; 10 beds) and the Department of Surgical Intensive Care Medicine (SIC; 12 beds).

Microbial contamination of air was determined using the MAS-100 microbial air monitoring system (Merck). At each department, samples were taken from a total of 10 sites. In all cases, 100 liters of indoor air were collected over a period of 1 minute.

Microbial contamination of surfaces was assessed using sterile swabs moistened with sterile saline. Samples were collected from a 10 × 10 cm area in two directions perpendicular to each other. In the case of sink and washbasin drains, samples were collected from the inner surfaces. At each department, 30 different surfaces were assessed (window boards, bed rails, infusion pump control panels, window blinds, sink drain, washbasin drain, rinse solution, tube stand).

Health care workers' nasal mucosa was examined with a single swab of both nostrils. Samples were collected using sterile swabs moistened with sterile saline. Skin of the right hand without a glove was examined by swabbing the skin in places usually neglected during scrub-up, *i.e.* the thumb area and both the upper and lower sides of the interdigital spaces. Also in this case, samples were collected using sterile swabs moistened with sterile saline. At each department, 15 persons were examined.

Enterobacteriaceae were identified using standard microbiological methods including the ENTEROtest 16 (Erba-Lachema) and, in selected cases, the Phoenix automated system (BD Diagnostics) and the MALDI-TOF (Bruker Daltonics). The isolated bacteria were stored in a deep freezer at −80˚C using the ITEST Kryobanka B (ITEST plus). ESBL production was detected by the modified double-disk synergy test [11,12]. Phenotypic detection of AmpC beta-lactamases was carried out with the AmpC disk test with 3-aminophenylboronic acid [13, 14]. Genetic detection of beta-lactamases was performed using PCR and a set of specific primers for determining the presence of the bla_{TEM}, bla_{SHV}, $bla_{\text{CTX-M}}$ a bla_{AmpC} genes [15]. SHV-products were digested with restriction endonuclease *Nhe*I, TEM-positive amplicons with the *Mse*I, *Sau3*AI, *Msp*I and *Hph*I enzymes for identification of mutations extending the spectrum of activity [16,17]. To classify the present CTX-M beta-lactamases into individual groups, restriction analysis was performed using *Bse*DI endonuclease [18]. To compare similar or identical isolates, pulsed-field gel electrophoresis (PFGE) of DNA fragments was used [19]. The resulting restriction profiles were compared using the GelComparII software (Applied Maths).

For comparison, *Enterobacteriaceae* were also isolated from clinical samples (tracheal secretions, bronchoalveolar lavage, sputum, blood, urine, pus, aspiration specimens, wound secretions) collected from patients hospitalized at the AIC and SIC departments during the same period. The strains were selected in such a manner that from each patient, only one strain for each species was included, which was isolated as the first one from the particular material.

3. Results

Over the study period, air samples for the assessment of bacterial contamination were collected from 20 sites. In addition, 60 swabs were taken from surfaces and 60 from health care workers (swabs from both nostrils and the right hand without a glove). From a total of 140 samples, 18 were found to contain 21 bacterial isolates of the *Enterobacteriaceae* family (**Table 1**).

Phenotypic analysis revealed ESBL production in 4 isolates; the AmpC disk test was positive in 7 cases. Environmental ESBL- and AmpC-positive isolates including beta-lactamase types are summarized in **Table 2**.

ESBL- and AmpC-positive *Enterobacteriaceae* isolated from clinical samples collected from patients at the aforementioned intensive care departments over the study period are listed in **Table 3**.

ESBL- and AmpC-positive isolates from both the environment and clinical samples from hospitalized patients were subjected to genetic analysis aimed at the detection of individual beta-lactamase types. This revealed the presence of the bla_{TEM} gene in 7 isolates, bla_{SHV} gene in 10 isolates and $bla_{\text{CTX-M}}$ gene in 11 isolates. In 4 of the 10 genes carrying the bla_{SHV} gene,

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Table 1. Numbers and species of isolates of the *Enterobacteriaceae* family detected in the intensive care unit setting (Department of Anesthesiology and Intensive Care Medicine-AIC; Department of Surgical Intensive Care Medicine-SIC).

	Surface swabs		Personnel swabs	
Species	SIC	AIC	SIC	AIC
Citrobacter koseri	0	0	2	0
Escherichia coli	1	0	1	0
Enterobacter cloacae	0	3	0	0
Klebsiella oxytoca	1	1	1	0
Klebsiella pneumoniae	3	6	1	0
Proteus vulgaris	1	0	0	0
Total	6	10	5	0

restriction analysis detected mutations at position 238, associated with an extended spectrum of activity of the particular beta-lactamase. In the detected bla_{TEM} genes, mutations extending the spectrum of activity were not confirmed by analyzing restriction fragment length. All the detected $bla_{CTX\text{-}M}$ genes were members of the CTX-M-like-1 group. Genes encoding AmpC beta-lactamases were observed in 13 isolates. Most frequently, a gene for the EBC type was identified. In only one case, a 405-bp amplicon was found, corresponding with the DHA type.

ESBL- and AmpC-positive isolates were also subjected to restriction analysis of genome DNA followed by PFGE to determine similarity or identity. This comparison included strains of the same species isolated from the clinical samples collected from patients hospitalized at the respective centers in November to December 2010. Among 11 (6 environmental and 5 patient) *Klebsiella pneumoniae* isolates, PFGE identified four isolates with an identical restriction profile; the remaining 7 ones were genetically different. Identical isolates were also detected in *Enterobacter cloacae* (3/5). In both species, identical strains were only found among isolates from the hospital environment. In all cases, those were surface swabs. No similarity was found between environmental isolates and strains isolated from patients over the study period.

4. Discussion

The study documented the presence of ESBL- and AmpC-positive *Enterobacteriaceae* in two intensive care units of the UHO. It is well known that in such types of departments, an increased use of antimicrobial agents is necessary, with selection pressure resulting in a high proportion of resistant strains. Of special importance are hygiene measures that are strictly adhered to in order to prevent the development of nosocomial infections and spread of resistant bacteria from the environment to patients and among them.

To emphasize the importance of adherence to hygiene practice and restriction of administration of third-generation cephalosporins, a study should be mentioned in which the above measures were necessitated by a high prevalence of ceftazidime-resistant *Klebsiella pneumoniae* strains at an intensive care unit. Such adherence

Table 2. ESBL- and AmpC-positive *Enterobacteriaceae* detected in anesthesiology intensive care and surgical intensive care settings.

Code	Species	Sample type		Dept.	Phenotype	BL type
M1	*Klebsiella pneumoniae*	Surface swabs	Window board at the patient's head	SIC	ESBL	CTX-M, SHV, TEM
M2	*Klebsiella oxytoca*	Surface swabs	Sink drain	SIC	ESBL	CTX-M, TEM
M3	*Klebsiella pneumoniae*	Surface swabs	Washbasin drain, Room 6	AIC	AmpC	EBC
M4	*Klebsiella pneumoniae*	Surface swabs	Washbasin drain, Room 7	AIC	AmpC	EBC
M5	*Enterobacter cloacae*	Surface swabs	Rinse solution, Room 6	AIC	AmpC	EBC
M6	*Enterobacter cloacae*	Surface swabs	Rinse solution, Bed 8	AIC	AmpC	EBC
M8	*Klebsiella pneumoniae*	Surface swabs	Bed rails, Bed 8	AIC	ESBL	CTX-M, SHV*
M10	*Enterobacter cloacae*	Surface swabs	Tube stand, Bed 8	AIC	AmpC	EBC
M11	*Klebsiella pneumoniae*	Surface swabs	Rinse solution, Bed 9	AIC	AmpC	EBC
M12	*Klebsiella pneumoniae*	Surface swabs	Dosing pump control panel, Bed 9	AIC	AmpC	EBC
M13	*Klebsiella oxytoca*	Surface swabs	Sink drain, Room 5	AIC	ESBL	CTX-M, TEM

Legend: Dept.: Department; BL: Beta-lactamase; *Mutation extending the spectrum of activity detected by restriction analysis; AIC: Department of Anesthesiology and Intensive Care Medicine; SIC: Department of Surgical Intensive Care Medicine.

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Table 3. ESBL- and AmpC-positive patient isolates.

Code	Species	Type of material	Dept.	Phenotype	BL type
M14	*Escherichia coli*	Mouth swab	AIC	ESBL	SHV*
M16	*Klebsiella pneumoniae*	Blood culture	SIC	ESBL	CTX-M, SHV
M17	*Klebsiella pneumoniae*	Wound swab	SIC	ESBL	CTX-M, SHV
M18	*Escherichia coli*	Urine	AIC	ESBL	CTX-M, TEM
M19	*Serratia marcescens*	Secretion	AIC	ESBL	SHV*
M20	*Klebsiella pneumoniae*	Cannule	AIC	ESBL	SHV*
M21	*Escherichia coli*	Urine	AIC	ESBL	CTX-M
M22	*Enterobacter cloacae*	Mouth swab	AIC	AmpC	EBC
M23	*Enterobacter cloacae*	Bronchoalveolar lavage	SIC	AmpC	EBC
M24	*Enterobacter cloacae*	Secretion	AIC	AmpC	EBC
M25	*Enterobacter cloacae*	Secretion	AIC	AmpC	EBC
M26	*Klebsiella pneumoniae*	Urine	AIC	AmpC	DHA
M29	*Klebsiella pneumoniae*	Wound swab	AIC	ESBL	CTX-M, SHV, TEM
M30	*Escherichia coli*	Wound swab	SIC	ESBL	CTX-M, SHV, TEM
M32	*Escherichia coli*	Secretion	AIC	ESBL	CTX-M, SHV, TEM
M34	*Enterobacter cloacae*	Secretion	AIC	AmpC	EBC

Legend: Dept.: Department; BL: Beta-lactamase; AIC: Department of Anesthesiology and Intensive Care Medicine; SIC: Department of Surgical Intensive Care Medicine.

proved to be highly effective, with a 4-fold increase in susceptibility to ceftazidime in *Klebsiella pneumoniae* isolates [10]. Although restrictive and barrier measures were only enforced in the ICU, there was a slight increase in susceptibility throughout the hospital [10].

Although the results of restricted use of antibiotics is positive it may not always be that significant. Toltzis *et al*. reduced the administration of ceftazidime at an ICU which resulted in a decrease, albeit insignificant, in ceftazidime-resistant Gram-negative bacteria. In the case of AmpC-positive bacteria, however, the prevalence dropped significantly from 68% to 46% [20].

Dancer *et al*. tried to measure the effect of enhanced cleaning of a surgical ward, with an additional cleaner. They found that such enhanced cleaning was associated with a 33% reduction in microbial contamination of hand-touch sites. Enhanced cleaning saved the hospital 30 to 70 thousand pounds [21].

Our study revealed the presence of ESBL- and AmpC-positive *Enterobacteriaceae* in the ICU setting but no transmission of these strains to patients was noted. This suggests that hygiene precautions applied at such departments are adequate. However, the high rates of resistant *Enterobacteriaceae* in environmental swabs prove a marked resistance of these microorganisms to disinfection programs, a potential threat to patients. An international prospective study (Europe, Asia, Africa, North and South America, Australia) by Peterson provided alarming data: 31% (78 out of 253) of episodes of nosocomial bacteremia and 43% (30 out of 69) of episodes acquired at ICUs were caused by ESBL-producing bacteria. Previous administration of a beta-lactam antibiotic with an oxyimino group (cefuroxime, cefotaxime, ceftriaxone, ceftazidime and aztreonam) was associated with a higher risk of ESBL-positive bacteremia. In 7 out of 10 hospitals, interpatient transmission was also identified [22].

Our study did not detect ESBL- and/or AmpC-positive *Enterobacteriaceae* in health care workers. According to Paterson et al., however, such a result does not rule out colonization of staff members with other multiresistant bacteria. In their study, no staff member carried ESBL-producing *Escherichia coli* but 23% of physicians and 32% of nurses had positive culture results that yielded methicillin-resistant *Staphylococcus aureus* or vancomycin-resistant *Enterococcus* species [23].

It may be concluded that there are effective measures for the control of multiresistant *Enterobacteriaceae* in the hospital setting, namely rational antibiotic therapy, strict adherence to hygiene precautions and education of both health professionals and the general public. However, another essential component of prevention is careful monitoring of the prevalence of resistant bacteria not

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only in patients' clinical samples but also in the hospital environment, including genetic analysis of isolated strains.

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