UDC:616.24-022.7-085.33

GASTROINTESTINAL TRACT - RESERVOIR OF EXTENDED SPECTRUM B LACTAMASES PRODUCING STRAINS COLONIZING RESPIRATORY TRACT IN INFANTS

Popova Gorica[1], Tasik V[2], Boskovska K[1], IlievskaT[1]
[1]Institut for respiratory diseases in children Kozle, Skopje; [2]Clinic of Pediatric Diseases, Skopje, R. Macedonia

ABSTRACT

Introduction The useage of broad spectrum antibiotics leads to emergence of ESBL (extended spectrum β lactamases) gram - negative strains. Members of the normal gastrointestinal (GI) tract flora may cause endogenous disease if they reach tissues where they can not be tolerated.

Objectives The aims of this study are: to elaborate the prevalence of ESBL – producing strains obtained from patients with respiratory tract infections (RTIs), to investigate gastrointestinal colonization and possible endogenous reservoirs of infection, and to elaborate some epidemiological features of patients harboring ESBLs.

Methods Standard microbiological procedures were used for detecting bacteria, modificate triple disk diffusion test for detecting ESBLs, and disc diffusion test for measurement of antibiotic susceptibility

From 20 randomly selected patients with ESBLs cultivated from respiratory tract, stool samples and gastric contents were taken for detecting GIT colonization at the same time.

Results Of all enterobacteriaceae, ESBLs producers were 62.21% (214/344). Of all stool samples, 100% (20/20) were with predominant ESBLs gram - negative flora, and of all gastric samples in 90% (18/20) ESBLs gram- negatives were isolated, of which 94.4% (17/18) identified to the species level were identical to those isolated from respiratory tract.

Conclusion The prevalence of ESBLs isolated from respiratory tract in young patients is increasing. It seems that gram-negative ESBL-producers isolated from respiratory tract were indigenous gastrointestinal tract flora. Some epidemiological findings suggest possible risk factor for translocation of microbiological flora.

Keywords: ESBL strains, indigenous microbiological flora, translocation

INTRODUCTION

Beta-lactams are a huge group of antibiotics which includes: penicillins, cephalosporins, monobactams and carbapenems. The presence of beta – lactam ring in their structure is common for these antibiotics [1, 2].

Members of the family *Enterobacteriaceae* usually produce plasmid coded enzymes known as beta lactamases (for example TEM-1, TEM-2 and SHV-1). The beta lactamases induce resistance to penicillins, in lower percentage to first generation cephalosprins, but they do not induce resistance to third generation cephalosporins [3]. More than 90% of ampicillin resistance among *E.coli* is because of TEM-1 production [3].

In the middle of the 1980s, a noval group of enzymes, called *e*xtended *s*pectrum *b*eta lactamases (ESBL), was detected. These enzymes evolved by mutation of parenteral genes for TEM-1, TEM-2 and SHV-1. These mutations lead to amino-acid changes near the active center of the enzymes, which enables them to hydrolyze the oxyimono-cephalosporins (cefotaxime, ceftriaxone, ceftazidime) and monobactams (aztreonam) [3, 4, 5]. According to Bush, Jakoby and Medeiros classification, ESBL enzymes are defined as beta-lactamases capable of hydrolyzing oxyimono-cephalosporins (third generation cephalosporins) and they are inhibited by beta-lactamases inhibitors, for example clavulanic acid. [4, 5, 6].

CTX – M is a recently described group of ESBL enzymes [7, 8, 9]. These enzymes successfully hydrolyse cefotaxime, but are less successful in hydrolyzing cefatzidime (the reason behind its given name) [7, 8]. CTX - M genes are located on plasmids with size from 7 to 260 kb [7]. These genes are derived from the *Kluyvera spp*. chromosome which is a saprophytic species [10], an example for horizontal gene transfer. CTX – M are reported from different parts of the world and it seems that these are the most prevalent ESBL enzymes nowadays.

Plasmids are extarchromosomal gene material and they are replicated independently of the chromosome. Besides ESBL genes, other genese responsible for the resistance to other groups of antibiotics (for example aminoglycosides) is located on the plasmids and because of these antibiotic choices for ESBL infection treatment sometimes is extremely limited [5]. These genetic elements can easily be transmitted by conjugation from one to another bacterial cell and this process overcomes the frame of bacterial species [5].

Gastrointestinal tract (GUT) is a complex eco - system which includes aproximatly 800-1000 different bacterial species [11]. In GUT this majority of bacterial species function together in many complex and different physiological processes. Furthermore, commensal GUT flora in the same time interacts with the host immune cells and host enterocele [11].

The diversity of bacterial eco – system is relatively poor in infancy, but later it reaches complexity and it stays relatively stabile during the life [12, 13, 14, 15]. Ecological balance between commensal GUT flora and human as a host may be destrubed by many factors. The most dramatic disturbance is a result of antimicrobial administration [16, 17, 18]. Antibiotic therapy is given to destroy bacteria cousing illness, but in the same time it effects commensal GUT flora. This is a side effect and it depends of many factors such as: type of antibiotic, its spectrum of activity, dose of antibiotic, length of antibiotic treatment and antibiotics pharmacokinetics and pharmacodynamics propartis. If the parenteraly given antibiotic is secreted in greater amount through intestinal mucosa, or if the antibiotic is excreted in active form through billiary system its inpact on commensal GUT flora will be greater [17, 19, 20]. Mainly this impact is on density of normal GUT flora and occurs of resistant strains [17]. This disturbace of normal GUT flora may lead to many pathological conditions in the human host [21, 22].

Members of the normal GUT flora may cause endogenous disease if they reach tissues where they can not be tolerated [23,24].

Objectives

The objectives of this study was to elaborate the prevalence of ESBL producing strains isolated from patients with respiratory tract infections. To elaborate gastrointestinal tract colonization as endogenous rezervour of infection. To elaborate some epidemiological features of patients harboring ESBL producing strain.

MATERIALS AND METHODS

For this study, samples from hospitalized patients in period from Januray 2014 to November 2014 were used. All patients were aged from 0 to 18 years and hospitalized in The Institute for respiratory deseases in children in Skopje, because of severity of infections of the respiratory tract. 268 strains of *Escherichia coli* and 76 *Klebsiella pneumoniae* were isolated and identificated from samples as sputum and/or tracheal aspirat, using standard microbiological procedures. For isolation and identification of bacteria, blood agar plate (Oxoid, UK), chromogenic agar plate – UTI (Oxoid, UK), ChromoID ESBL agar (bioMerieux, France) – which is chromogenic selective medium for ESBL scrinning were used. For investigating of biochemical activity and mobility of bacteria IMVC (indol, metil rot, Voges-Proskauer, citrate) mediums were used. They were home prepared from dry supstances made in Merck, Germany.

Disc diffusion test was used for measurement of antimicrobial susceptibility. For this purpose bacterial suspensions with turbidity of 0.5 McFarlands were prepeard and than inoculated on Mueller-Hintons agars wich were 4 mm in depth. On the surface of the plates, antimicrobial discs were placed manually and the distance between the discs was approximatly 3cm. The following discs (Oxid, UK) were used: amoxicillin/clavulanic acid (20/10μg), cefotaxime (30μg), ceftazidime (30μg), cefoxitin (30μg), cefepime (30μg), imipenem (10μg), ciprofloxacin (5μg), trimethoprim-sulfamethoxazole (1.25/23.75μg), amikacin (30μg), gentamicin (10μg). The plates were incubated at aerobic conditions, at temperature of 37^{o}C, during 18 – 24 hours. The interpretation of the tests was according CLSI (Clinical Laboratory Standard Institute) recommendations, measuring the zone of inhibition around the antibiotic discs. Strains wich were resistant towards some of the third generation cephalosporins were selected as suspect ESBL producers. These strains were ongoing for phenotypic testing for ESBL, so, modificated triple disc diffusion test (suggested by Jarlier et al.) was performed. This test is based on synergy acting between third generation cepfalosporins and cavulanic acid. The susceptibility disc, containing amoxicillin-clavulanate, was placed in proximity to discs containing ceftazidim (CAZ) and cefotaxim (CTX). Enhancement of the zone of inhibition of the CAZ and CTX caused by the synergy of the clavulanate in the amoxicillin-clavulanate disc indicates the presence of Bush group 2be enzyme. Using this method, 214 ESBL positive strains were selected of wich *Escherichia coli* - 190 and *Klebsiella pneumoniae* - 24. All selected ESBL-positive strains were sussceptible to cefoxitin (30 μg) and imipenem (10 μg). This excluded the presence of AmpC – beta – lactamases (wich are capable of hydrolyzing cefoxitin and they are not inhibited by clavulanic acid), and the presens of carbapenemases (imipenem is supstrat of carbapenemases acting).

Gastric lavation and feces from 20 randomly selected patients with ESBL strain isolated from sputum and/or tracheal aspirat were taken for microbiological analyses at the same time. The samples from the digestive system were seeded on blood agar plate (Oxoid) and incubated aerobicly at 37^{o}C during 18-24 hours. After the incubation time for futher analyses only the Gram – negative strains which were dominant on the plate were undertaken. The same principle (previously explained) was used for detection of ESBL ensymes. Resistotypization was done by using the same antibiotic discks in disc diffusion tests for isolates from respiratory tract and from digestive system.

In purpose to identify the possible risk factors for infection or colonisation with ESBL positive strains, retrogradely 50 records fom patients with ESBL isolates from sputum or tracheal aspirat were analised.

RESULTS

In a period from Januray 2014 to November 2014, of all Grams - negatives isolated from sputum/tracheal aspirat, ESBL producers were 62.21% (214/344). Of all isolated *Escherichia coli*, ESBL - positive were 70.89% (190/268), and of all isolated *Klebsiella pneumoniae*, ESBL- positive were 37.58% (24/76). Of (n=20) patients with ESBL- strain isolated from sputum / tracheal aspirat, samples from digestive system (feces and gastric lavat) were taken for microbiological analysis. In 100% (20/20) ESBL – positive Gram – negative strains (as dominant flora) were isolated from aerobicly cultivated feceses (fig. 1.).

In 90% (18/20) ESBL – positive Gram – negative strains were isolated from aerobicly cultivated gastric lavations.

In those (n=17) cases where the same strain (*Escherichia coli*) was isolated from sputum and gastric lavat, resistotypization was done and in 88.2% (15/17) the same resistotype was identified (fig. 2, fig. 3).

Retrogradely analised records of hospitalised patients (n=50) with ESBL – positive strain isolated from respiratory tract, showed that middle age of the patients was 5,6 months (from 1 to 10 months). In 40% (20/50) there was a previous hospitalization (in the last four mounts).

Before hospitalisation, 38% (19/50) had been treated with perorally given cephalosporins (cefixime, cefaclor, cefuroxime, cefpodoxime). All patients (n=50) during hospiatlisation were treated with parenteral cephalosporins and middle time of antibiotic treatmen before the samples for microbiological analysis was taken was 2.58 days.

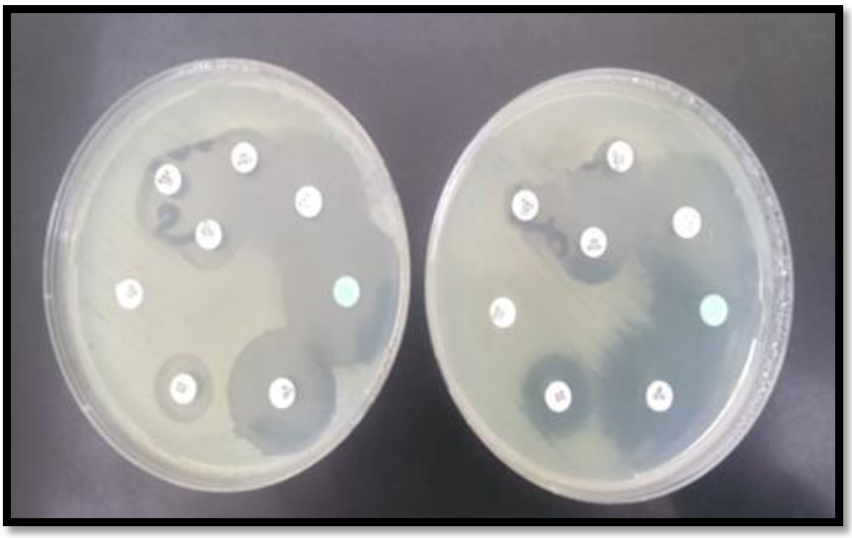

Fig. 1. Resistotyping of two strains (*Escherichia coli*) isolated from sputum and feces in the same patient

Fig. 2. Resistotyping of two strains (*Escherichia coli*) isolated from sputum and gastric lavat in the same patient

Fig. 3. Comparison between resistotypes isolated from sputum, feces and gastric lavat in the same patient

DISCUSSION

The intoduction of 3rd generation cephalosporins in clinical practice in the early 80s means a successful fight agains bacterial resistance conditioned by production of beta lactamases.

In 1983 the first plasmid-coded beta- lacatamase capeble to hydrolyse the 3rd generation cephalosporins was puplished [23]. Since then more than 220 TEM and 180 SHV types of beta –lactamases with caracteristics of ESBL have been detected. World wide, new types of beta-lactamases continually are detected and they are registrated in few databases [24, 25].

Since their first appearance, ESBL producing strains have dramatically spread [26, 27, 28, 29, 30].

There are more relevant studies about the inpact of parenteral given antibiotics on commensal GUT flora [31, 32, 33, 34, 35]. One of the unwanted effects is appearance of resistant strains, including ESBL producing gram negatives. This aspect is proven by many clinical studies and many experimental studies on animal models [36, 37, 38, 39].

On the other hand, there are studies which attest the preventive role of orraly given beta lactamases, capable to hydrolyse the part of parenteraly given antibiotic which is secreted in the GUT [36, 37, 39].

These findings are hipoteticly very interesting in purpose to prevent GUT flora by the acting of pareteraly given antibiotics.

In our study as a risck factor for colonization/infection of respiratory tract with gram- negatives, including ESBL starains is a small age (under the age of one).

Acid gastric content is a huge barrier for bacteria, but according Mitchella's and her coworkers's research, gastric content in infancy has different biochemical characteristics [41]. Milk is the basis of infancy feeding and milk has near neutral pH with little changes during lactation. Besides the pH value, milk has significantly specified puffer capacity [42].

Mitchella and coworkers were surprised because of the small reflux index measured in infants with clinical manifestation of reflux diseases. The explanation is that a long period of time during pH monitoring, the gastric content had pH >4 (with these pH values reflux can not be detected) and this was connected with the feeding of milk.

Besides the pH values of gastric content, the length of oesophagus, immaturity of gastroesophageal sphincter and horizontal position are possible risk factors for bacterial translocation.

Possibility of bacterial translocation is supported by our microbilogical findings with high percent of identical strains and resistotypes isolated from both – GUT and respiratory tract.

There is a need for future molecular researches in order to detect wich is the molecular similarity between the strains isolated from both – GUT and respiratory tract in the same patient [43, 44, 45].

CONCLUSION

There is a high prevalence of ESBL producing starins isolated from respiratory tract in small children (under the age of one). Using 3rd generation cephalosprins is a major risk factor for appearance of ESBL producing strains It seems that gram-negative ESBL-producers isolated from respiratory tract were indigenous gastrointestinal tract flora and GUT is their main reservoir . Small age is a risik factor for translocation of bacteria, enabling the colonisation of the respiratory tract.

REFERENCES:

1. Jawetz E, Melnick JL, Adelberg AE. Medicinska mikrobiologija. 20 izd. Belgrad: Savremena administracija, 1998.
2. Cazzola M, Blasi F, Ewing S. Antibiotics and the lung. Eur Respir Mon. 28th ed. Sheffield: Europian Respiratory Society Journals Ltd, 2004.
3. Bredford PA. Extended spectrum ß lactamases in the 21st century: characterization, epidemiology and detection of this important resistance threat. Clin Microbiol Rev 2001; 48:933-51.
4. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended broad spectrum beta lactamases conferring transferable resistance to newer beta lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. Rev Infect Dis 1988; 10:867-76.
5. Paterson LD, Bonomo AR. Extended spectrum beta lactamases: a clinical update. Cli Microbiol Rev 2005; 18(4):657-86.
6. Bush K, Jacoby GA. Update functional classification sheme of β – lactamases. Antimicrob Agents Chemother 2010; 54(3):969-976.
7. Bonnet R. Growing group of extended spectrum: the CTX-M enzymes. Antimicrob Agent Chemother 2004; 48:1-14.
8. Lewis JS, Herrera M, Wickes B, Patterson JE, Jorgensen JH. First Report of the emergence of CTX-M-type extended-spectrum β-Lactamases (ESBLs) as the predominant ESBL isolated in a U.S. health care system. Antimicrob Agents Chemother. 2008; 52(2): 810
9. Alobwede I, Mzali FH, Livermore DM, Hentige J, Todd N, Hawkey PM. CTX-M extended-spectrum beta-lactamases arrives in UK. J Antimicrob Chemother 2003; 51: 470-1.
10. Humeniuk C, Arlet G, Gautier V, Grimont P, Labia R, Philippon A. Beta-lactamases of Kluyvere ascorbata probabl progenitors of some plasmid – encoded CTX-M types. Antimicrob Agent Chemother 2002;46:3045-49.
11. Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host bacterial mutualism in the human intestine. Science 2005;307:1915-20.
12. Penderes J, Thijs C, Vink C et al. Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics 2006;118(2):511-21.
13. Jernberg C, Lofmark S, Edlund C, Jansson JK. Long – term ecological impacts of antibiotic administration on the human intestinal microbiota. Microbiology 2010;156:3216-23.
14. Zoeteudal EG, Akkermans AD, De Vos WN. Temperature gradient gel electrophoresis analysis of 16SrRNA from human fecal samples reveals stable and host – specific communities of active bacteria. Appl Environ Microbiol 2009;64:3854 - 9.
15. Qin J, Zi R, Raes J et al. A human gat microbial gene cataloque established by metagenomic sequencing. Nature 2010;464:59-65.
16. Rolan JM. Food and human gut as reservoirs of transferable antibiotic resistance encoding genes. Front Microbiol 2013; 4:173
17. 17.de la Cochetiere MF, Durand T, Lepage P, Bourreille A, Galmiche JP, Dore J. Resilience of the dominant human fecal microbiota upon short-course antibiotic challenge. J Clin Microbiol 2005;43:5588-92.
18. Perez-Cobas AE, Gosalbes MJ, Friedrichs A et al. Gut microbiota disturbance during antibiotic therapy: a multi-omic approach. Gut 2013;62:1591-601.
19. Sullivan A, Edlund C, Nord CE. Effect of antimicrobial agents on the ecological balance of human microflora. Lancet Infect Dis 2001;1:101-14.
20. Karami N, Martner A, Enne VI et al.Transfer of ampicillin resistance gene between two *Escherichia coli* strains in the bowel microbiota of an infant treated with antibiotics. J Antimicrob Chemother 2007; 60: 1142-5.
21. Young VB, Schmidt TM. Antibiotic-associated diarrhea accompanied by large-scale alterations in the composition of the fecal microbiota. J Clin Microbiol 2004;42:1203–6.
22. Tlaskalova-Hogenova H, Stepankova R, Hudcovic T, et al. Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases. Immunol Lett. 2004;93(2-3):97-108.
23. Knothe H, Shah P, Krcmery V, Antal M, Mitsuhashi S. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. Infection. 1983;11**:**315-7.

24. Bush K, Jacoby GA. Beta – lactamase classification and amino acid sequences for TEM, SHV and OXA extended spectrum and inhibitor resistant enzymes. http://www.lahey.org/studies.
25. Abhishikha Srivastava, Neelja Singhal, Manisha Goel, Jugsharan Singh Virdi, Manish Kumar. CBMAR: a comprehensive β-lactamase molecular annotation resource. Database (Oxford). 2014; 2014: bau111 http://14.139.227.92/mkumar/lactamasedb.
26. Albertini MT, Benoit C, Berardi L, et all. Surveillance of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Enterobacteriaceae* producing extended-spectrum beta-lactamase (ESBLE) in Northern France: a five-year multicentre incidence study. J Hosp Infect 2002;52**:**107-13.
27. Fedler KA, Biedenbach DJ, Jones RN. Assessment of pathogen frequency and resistance patterns among pediatric patient isolates: report from the 2004 SENTRY Antimicrobial Surveillance Program on 3 continents. Diagn Microbiol Infect Dis. 2006;56(4):427-36.
28. Chandramohan L, Revell PA. Prevalence and Molecular Characterization of Extended-Spectrum-β-lactamase producing Enterobacteriaceae in a pediatric patient population. Antimicrob Agents Chemother. 2012;56(9):4765-70.
29. Blaschke AJ, Korgenski K, Daly JA et al. Extended-spectrum beta-lactamase-producing pathogens in a children ˜s hospital: a five-year experience. Am J Infect Control 2009;37(6):435-41.
30. Gupt A, Ampofo K, Rubenstein D, Saiman L. Extended spectrum beta lactamase producing *Klebsiella pneumoniae* infections: a review of the literature. Journal of Perinatology 2003;23:439-43.
31. Christiaens G, Ciccarell Y, Damas P, Hayette MP, Melin P, Nys M, De Mol P. Prospective survey of digestive tract colonization with Enterobacteriaceae that produce extended-spectrum β lactamases in intensive care units. J. Hosp. Infect 2006;62:386-8.
32. Lindgren M, Lofmark S, Edlund C, Huovinen P, Jalava J. Prolonged impact of a one-week course of clindamycin on *Enterococcus* spp. in human normal microbiota. Scand J Infect Dis 2009;41:215–9.
33. Nyberg S D, Osterblad M, Hakanen A J, Lofmark S, Edlund C, Huovinen P, Jalava J. Long-term antimicrobial resistance in *Escherichia coli* from human intestinal microbiota after administration of clindamycin. Scand J Infect Dis 2007;39:514–20.
34. Valverde A, Coque TM, Sanches-Moreno MP, et al. Dramatic increase in prevalence of fecal carriage of extended spectrum beta lactamase producing *Enterobacteriaceae* during non outbreak situations in Spain. J Clin Microbiol 2004;42(10):4769-75.
35. Quigley EM, Quera R. Small intestinal bacterial overgrowth:roles of antibiotics, prebiotics and probiotics. Gastroenterology 2006;130:S79-S90.
36. Harmoinen J, Mentula S, Mentula S, et al. Orally administered targeted recombinant β-lactamase prevents ampicillin-induced selective pressure on the gut microbiota: a novel approach to reducing antimicrobial resistance. Antimicrob Agents Chemother 2004;48:75-9.
37. Tarkkanen AM, Heinonen T, Jog R, et all. P1A recombinant β-Lactamase prevents emergence of antimicrobial resistance in Gut microflora of healthy subjects during intravenous administration of Ampicillin Antimicrob. Agents Chemother. 2009; 53:6
38. Nicole JP, Usha S, Curtis JD. Effects of daptomycin, linezolid, and vancomycin on establishment of intestinal colonization with vancomycin-resistant enterococci and extended-spectrum beta-lactamase producing Klebsiella pneumoniae in Mice Antimicrob. Agents Chemother 2005; 49(8): 3513-6.
39. Stiefel U, Nerandzic MM, Koski P, Curtis J. Donskey. Orally administered beta-lactamase enzymes represent a novel strategy to prevent colonization by Clostridium difficile. J Antimicrob Chemother 2008; 62 (5):1105-8.
40. Curtis J. Donskey. The role of the intestinal tract as a reservoir and source for transmission of nosocomial pathogens. Clinical Infectious Diseases 2004;39(2): 219-26.
41. Mitchella DJ, McClurea BG, Tubmanb TRJ. Simultaneous monitoring of gastric and oesophageal pH reveals limitations of conventional oesophageal pH monitoring in milk fed infants. Arch Dis Child 2001; 84:273-6.
42. Morriss H F, Brewer DE, Spedale BS et al. Relationship of human milk pH during course of lactation to concentrations of citrate and fatty acids. Pediatrics 1986;78:458-464.
43. Healy M, Houn J, Lupski JR. Mycrobial DNA typing by automasted repetitive swquence based PSR. J Clin Microbiol 2005;43(1):199–207.
44. 44. Nemoy LL, Kotetishvili M, Tigno J, et al. Multilocus sequence typing versus pulsed-field gel lectrophoresis for characterization of extended-spectrum beta-lactamase-producing Escherichia coli isolates. J Clin Microbiol 2005;43(4):1776-81.
45. 45.Brolund A, Hæggman S, Edquist PJ, Gezelius L, Olsson-Liljequist B, Wisell KT, Giske CG. The DiversiLab system versus pulsed-field gel electrophoresis: characterisation of extended spectrum β-lactamase producing Escherichia coli and Klebsiella pneumoniae. J Microbiol Methods 2010;83(2):224-30.