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Full Length Research Paper

CTX-M-15-producing *Enterobacteriaceae* isolates causing bloodstream infections at the Beni-Messous hospital in Algiers (Algeria)

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Abstract

The purpose of this was to characterize the ESBL produced by multiresistant *Enterobacteriaceae* isolates and their sensitivity profile recovered from children admitted at the pediatric ward of Beni-Messous hospital (Algeria) during the period May 2008 to August 2010 at the Beni-Messous hospital in Algiers (Algeria). 31 ESBL-producing *Enterobacteriaceae* isolates were tested towards 21 antimicrobial agents. PCR were used to determine the genetic determinants responsible for the ESBL phenotypes. All isolates were found resistant to ticarcillin, ticarcillin-clavulanate, piperacillin, amoxicillin-clavulanate, cefuroxime, aztreonam, ceftazidime, cefotaxime, cefepime and cefpirome. PCR and DNA sequencing identified these extended-spectrum β-lactamases as CTX-M-15 in all isolates.

Keywords: Bloodstream infections, ESBL, *Enterobacteriaceae*, Gram negative bacteremia, Antibiotic resistance, Algeria.

INTRODUCTION

Bloodstream infections (BSI) are the most common form among all hospital acquired infection in pediatric patient, and these type of infection are responsible for approximately 10% to 30% of the all cases. Currently, Gram-negative bacteria are most important agents that are involved in pediatric bloodstream infections and such strains responsible for approximately 24% to 50% of hospital acquired infection cases (Arnoni et al., 2007).

Resistance to third-generation cephalosporins in these isolates is largely due to the production of extended-spectrum β-lactamase (ESBL) enzymes, which hydrolyse oxyimino-cephalosporins that are inhibited by clavulanic acid (Dashti et al., 2010).

Organisms (especially *Klebsiella* spp. and *Escherichia coli*) which are producing extended-spectrum β-lactamases are clinically relevant and important causes for the failure of therapy with cephalosporins, especially when they are mainly responsible for bloodstream-associated infections (Pitout et al., 2009).

Bacteremia caused by ESBL-producing members of the family *Enterobacteriaceae* was associated with adverse outcomes, including higher rates of mortality, increased lengths of hospital stays, delays in the time to the receipt of the appropriate therapy, the discharge of a higher proportion of patients to chronic care, and significantly higher costs, compared to the outcomes for patients with bacteremia caused by non-ESBL producers (Pitout et al., 2009).

The goal of this study was to characterize the ESBL produced by multiresistant *Enterobacteriaceae* isolates and their sensitivity profile recovered from children admitted at the pediatric ward of Beni-Messous hospital (Algeria).

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MATERIALS AND METHODS

Patients

The study was conducted over a period of 27 months from May 2008 to August 2010 to all patients admitted at pediatric ward at Beni-Messous hospital in Algiers (Algeria). Nosocomial blood stream infections were defined on the basis of these criteria:

Criterion 1: Patient has a recognized pathogen cultured from one or more blood cultures and the organism cultured from blood is not related to an infection at another site. Criterion 2: Patient has at least one of the following signs or symptoms: fever (> 38°C), chills, or hypotension. The Signs and symptoms of infection appear 48 hours to four days after admission, and there are no signs or symptoms of infection at the time of admission, proven by history and clinical examination (Garner et al., 1988; Ahmed et al., 2009).

Collection and Processing of Blood samples

5 to 10 ml of venous blood was collected from patients using sterile syringes. Blood samples were inoculated immediately under complete aseptic conditions into bottles containing 50 ml of brain heart infusion broth (Ahmed et al., 2009).

The blood culture bottles were incubated aerobically at 37°C for 7 days. The bottles were examined daily for evidence of bacterial growth as haemolysis, gas production or turbidity. Subcultures using sterile syringes were done on blood agar and Mac Conkey's agar daily for 7 days before reporting blood cultures as negative (Ahmed et al., 2009).

Enterobacteriaceae isolates were identified by the API 20E identification system following the manufacturer's recommendations (BioMérieux, Marcy l'Étoile, France). The isolates were stored at -80 °C in 15% glycerol (v/v) in trypticase-soy broth. They were subcultured on trypticase-soy agar and incubated aerobically at 37°C for 24 h.

Antimicrobial Susceptibility testing

Antibiotic susceptibility testing was performed by the disc-diffusion method on Mueller–Hinton agar, which was incubated at 37 °C for 18 h. The results were interpreted according to the recommendations of the Antibiogram Committee of the French Society for Microbiology (http://www.sfm.asso.fr). The following antibiotics were tested: aztreonam, ticarcillin, piperacillin, amoxicillin-clavulanate, ticarcillin-clavulanate, cefoxitin, cefpirome, cefepime, piperacillin-tazobactam, cefuroxime, imipenem, cefotaxime, ceftazidime, tobramycin, amikacin, gentamicin, co-tromixazol, ciprofloxacin, tetracycline and chloramphenicol. *Escherichia coli* strain ATCC 25922 was used for quality control.

Detection of Extended Spectrum β-Lactamases

Extended-Spectrum β-Lactamase (ESBL) production was detected by a double-disk synergy test (DDST) and was performed by placing disks of ceftazidime, cefotaxime, cefepime and aztreonam at a distance of 20mm (centre to centre) from a disk with amoxicillin/clavulanic acid (20/10 µg) (Jarlier et al., 1988). In *Enterobacteriaceae* species naturally producing cephalosporinase (AmpC), the inhibition of AmpC β-lactamases by cloxacillin (200 mg/l) is used in ESBL confirmation tests (Brasme *et* al., 2007). ESBL production was considered positive when an enhanced zone of inhibition was visible between the β-lactam and β-lactamase inhibitor-containing discs.

The presence of β-lactamase genes was investigated by PCR assays. Primers used to amplify bla_{CTX-M} gene were CTX-M1A (CTTCCAGAATAAGGAATC) / CTX-M-1-647R (CCTTTCATCCATGTCACCA) and CTX-M-1-406-F (GTGGCGATGAATAAGCTGA)/ CTX-M1B (CCGTTTCCGCTATTACAA) (Brasme et al., 2007). PCR conditions for the CTX-M gene comprised an initial an initial denaturation at 94°C for 5 min, denaturation at 94°C for 30s, annealing at 52°C for 30s, and elongation at 72°C for 30s, repeated for 30 cycles; and a final extension at 72°C for 7 min.

RESULTS

During the 3 years of surveillance (May 2008 to August 2010), a total of 31 children with incident bloodstream infections due to ESBL-producing *Enterobacteriaceae* isolates were identified at the pediatric ward of Beni-Messous hospital in Algiers (Algeria). All isolates were classified as hospital-acquired infections. Of the 31 isolates included in this study, 23 isolates were identified as *Klebsiella pneumoniae*, 5 as *Enterobacter cloacae* and 3 as *Escherichia coli*. 16 isolates were recovered in 2008, 9 isolates in 2010 and 6 in 2009. 16 patients (51.6%) were females.

The rates of resistance of the *Enterobacteriaceae isolates* to the antimicrobials agents tested are given in Figure 1. All isolates exhibited decreased susceptibilities to β-lactams antibiotics, gentamycin and tobramycine. They remained susceptible to imipenem, ticarcillin-clavulanate (except *E. cloacae* isolates), amoxicillin-clavulanate (except *E. cloacae* isolates), cefoxitin (except *E. cloacae* isolates) and piperacillin-tazobactam.

The DDS-test was positive for all of these isolates Figure 2. Of the 31 ESBL-producing *Enterobacteriaceae* isolates recovered from blood, all produced CTX-M-15 ESBL.

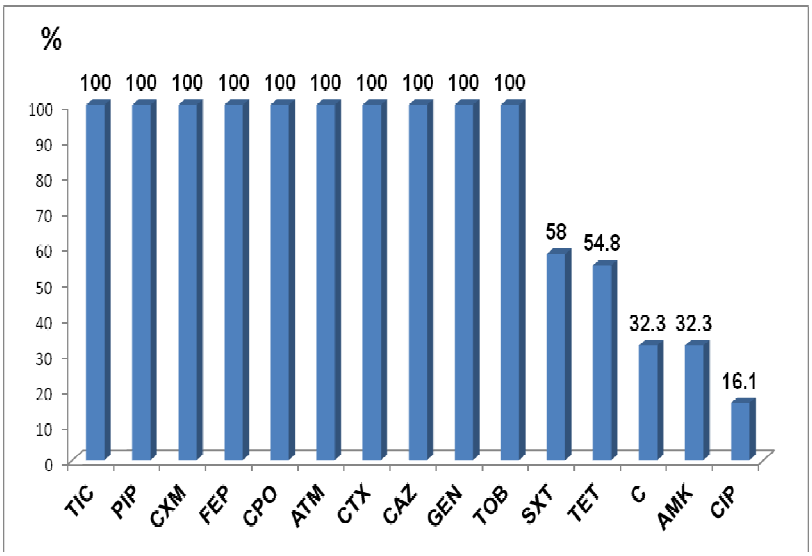

Figure 1. Rates of antibiotic resistance of Enterobacteriaceae isolates recovered from bloodstream infection

Legend: TIC: ticarcillin, PIP: piperacillin, CXM: cefuroxime, FEP: cefepime, CPO: cefprirom, ATM: aztreonam, CTX: cefotaxim, CAZ: ceftazidime, GEN: gentamicin, TOB: tobramycin, SXT: co-tromixazol, TET: tetracycline, C: chloramphenicol, AMK: amikacine, and CIP: ciprofloxacin.

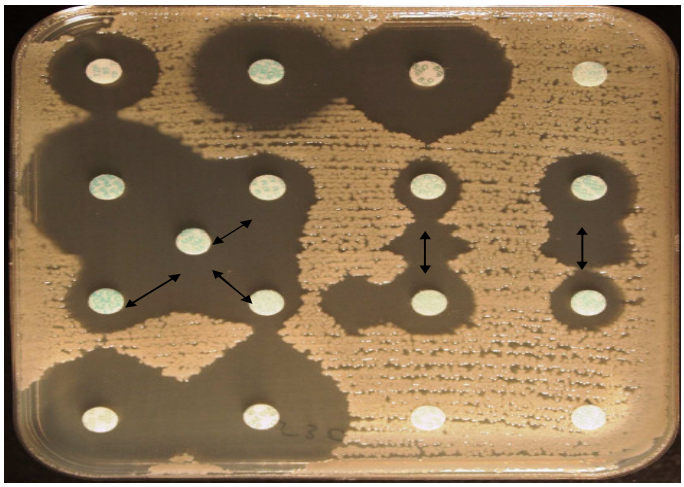

Figure 2. Results of a DDS-test showing a positive ESBL reaction between amoxicillin–clavulanate and β-lactam discs (the arrows show synergy between discs)

DISCUSSION

In the present study, *Klebsiella pneumoniae* producing ESBL was the most commonly isolated *Enterobacteriaceae* organism, followed by *Enterobacter cloacae and E coli*. These results are similar to those of other recent nosocomial BSI studies where these organisms have been among the leading Gram-negative pathogens. Antibiotic resistance is a growing problem in hospitals everywhere (Ahmed et al., 2009).

With the widespread use of extended-spectrum cephalosporins throughout the world, isolates that produce ESBLs have been detected on every inhabited continent. The prevalence and relative distribution of

ESBLs vary depending on the facility and the level of care taken to control nosocomial BSI. These factors also vary with geographic location and time (Shah et al., 2004). These enzymes are most commonly found in *K. pneumoniae*, but they are increasingly found in *E. coli*, *Proteus mirabilis* and other gram negative bacilli (Kim al., 2002).

In the present study, the type of β-lactamase gene was determined among *Enterobacteriaceae isolates* by using a polymerase chain reaction, which showed that CTX-15 was the unique type of ESBL produced by these organisms. Until the late 1990s, surveys of extended-spectrum β-lactamases (ESBLs) almost exclusively found TEM and SHV enzyme variants, and largely found these in Klebsiella spp. These patterns have now changed dramatically, with CTX-M enzymes replacing TEM and SHV mutants as the predominant ESBLs in many countries, with *Escherichia coli* joining *K. pneumoniae* as a major host. CTX-M enzymes are more active against cefotaxime and ceftriaxone than ceftazidime, but point mutations can increase activity against ceftazidime; thus CTX-M-15 differ from CTX-M-3 only by Asp-240→Gly substitutions, but are 100-fold more active against ceftazidime. (Livermore et al., 2007). CTX-M ESBL was reported in bloodstream infections by many authors. For example, Dashti et al., demonstrated the transmission of a clone of CTX-M-15-producing *K. pneumoniae* among neonates and healthcare workers in Kuwait (Dashti et al., 2010). Lee et al., detected $bla_{(CTX-M}$ in 90.9% of *E. coli* isolates and 20.9% of *K. pneumoniae* isolates with the ESBL phenotype. CTX-M-15 was the 2nd most common type of CTX-M ESBLs in *E. coli* (n=22), but it was not detected in *K. pneumoniae* (Lee et al., 2009). In Algeria, CTX-M-15 and CTX-M-3 have been identified in nosocomial isolates of various *Enterobacteriaceae*, such as *E. coli*, *K. pneumoniae* and *E. cloacae* (Touati al., 2006; Iabadene et al, 2008; Messai et al., 2008; Ramdani-Bouguessa et al., 2011). However, no reports for the presence of these enzymes in bloodstream infections have been published in Algeria.

The optimal therapy for infections caused by ESBL-producing members of the family *Enterobacteriaceae* has yet to be established. Therapeutic options include β-lactamase inhibitor combinations with cephamycin, carbapenems, fluoroquinolones and aminoglycosides (Kim al., 2002). In our study, results of susceptibility testing revealed a limited group of effective antibiotics for the treatment of infections caused by these organisms. Carbapenems are the effective for treatment of these infections, whereas extended-spectrum penicillins and cephalosporins are not likely to be successful in treating these infections. All isolates were resistant to gentamicin, tobramycin and 32% isolates were resistant to amikacin, therefore, aminoglycosides are not recommended for the treatment of these infections. In addition, 16% of isolates were found to be resistant to ciprofloxacin. As mentioned by Zaoutis et *al.* this is a surprising finding because ciprofloxacin is rarely used in the routine clinical practices of our pediatric hospital (Zaoutis et al., 2011).

Infections caused by ESBL-*Enterobacteriaceae* species are of great concern for many reasons. Current methods of identification can result in the underestimation of the prevalence of these organisms. Finally, previous studies have shown that both adult and pediatric patients with ESBL-*Enterobacteriaceae* infections have an increased risk for clinical failure and death compared to patients with non-ESBL infections (Zaoutis et al., 2011).

The recently reported increase in CTX-M–producing *Enterobacteriaceae* in Algeria raises concern. Thus, an increase in CTX-M–producing isolates is likely to directly affect treatment, especially among children for whom use of fluoroquinolones is contraindicated. Unfortunatly, the results obtained in our study are limited, thus further epidemiological studies of bloodstream infections caused by *Enterobacteriaceae* in Algeria are recommended to determine risk factors for *Enterobacteriaceae* producing Extended-Spectrum β-Lactamase bloodstream Infection.

Bloodstream infections with multidrug resistant pathogens (especially ESBLs) are difficult to treat and are associated with increased mortality. Of all available anti-microbial agents, carbapenems are the most active and reliable treatment options for infections caused by ESBL isolates. Unfortunately, overuse of carbapenems may lead to the selection of Gram-negative organisms producing carbapenemase.

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REFERENCES

Ahmed SH, Daef EA, Badary MS, Mahmoud MA, Abd-Elsayed AA (2009). Nosocomial blood stream infection in intensive care units at Assiut University Hospitals (Upper Egypt) with special reference to extended spectrum beta-lactamase producing organisms. BMC. Res. Notes. 6: 76.

Antibiogram Committee of the French Microbiology Society. Report 2010. French Microbiology Society, Paris. P. 50.

Arnoni MV, Berezin EN, Martino MD (2007). Risk factors for nosocomial bloodstream infection caused by multidrug resistant gram-negative bacilli in pediatrics. Braz. J. Infect. Dis. 2: 267-271.

Brasme L, Nordmann P, Fidel F, Lartigue MF, Bajolet O, Poirel L, Forte D, Vernet-Garnier V, Madoux J, Reveil JC, Alba-Sauviat C, Baudinat I, Bineau P, Bouquigny-Saison C, Eloy C, Lafaurie C, Simeon D, Verquin JP, Noel F, Strady C, De Champs C (2007). Incidence of class A extended-spectrum beta-lactamases in Champagne-Ardenne (France): a 1 year prospective study. J. Antimicrob. Chemother. 60: 956-964.

Dashti AA, Jadaon MM, Gomaa HH, Noronha B, Udo EE (2010). Transmission of a *Klebsiella pneumoniae* clone harbouring genes for CTX-M-15-like and SHV-112 enzymes in a neonatal intensive care unit of a Kuwaiti hospital. J. Med. Microbiol. 59: 687-692

Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM (1988). CDC definitions for nosocomial infections. Am. J. Infect. Control. 16:128–140.

Iabadene H, Messai Y, Ammari H, Ramdani-Bouguessa N, Lounes S, Bakour R, Arlet, G (2008). Dissemination of ESBL and Qnr

determinants in *Enterobacter cloacae* in Algeria. J. Antimicrob. Chemother. 62: 133-136.
Jarlier V, Nicolas MH, Fournier G, Philippon A (1988). Extended-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. Rev. Infect. Dis. 10: 867-878.
Kim YK, Pai H, Lee HJ, Park SE, Choi EH, Kim J, Kim JH, Kim EC (2002). Bloodstream infections by extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in children: epidemiology and clinical outcome. Antimicrob. Agents. Chemother. 5: 1481-1491.
Lee SG, Jeong SH, Lee H, Kim CK, Lee Y, Koh E, Chong Y, Lee K (2009). Spread of CTX-M-type extended-spectrum beta-lactamases among bloodstream isolates of *Escherichia coli* and *Klebsiella pneumoniae* from a Korean hospital. Diagn. Microbiol. Infect. Dis. 63: 76-80.
Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G, Ayala J, Coque TM, Kern-Zdanowicz I, Luzzaro F, Poirel L, Woodford N (2007). CTX-M: changing the face of ESBLs in Europe. J. Antimicrob. Chemother. 59: 165-174.
Messai Y, Iabadene H, Benhassine T, Alouache S, Tazir M, Gautier V, Arlet G, Bakour R (2008). Prevalence and characterization of extended-spectrum beta-lactamases in *Klebsiella pneumoniae* in Algiers hospitals (Algeria). Pathol. Biol. 56: 319-325.
Pitout JD, Gregson DB, Campbell L, Laupland KB (2009). Molecular characteristics of extended-spectrum-beta-lactamase-producing *Escherichia coli* isolates causing bacteremia in the Calgary Health Region from 2000 to 2007: emergence of clone ST131 as a cause of community-acquired infections. Antimicrob. Agents. Chemother. 7: 2846-2845.
Ramdani-Bouguessa N, Manageiro V, Jones-Dias D, Ferreira E, Tazir M, Caniça M (2011). Role of SHV β-lactamase variants in resistance of clinical *Klebsiella pneumoniae* strains to β-lactams in an Algerian hospital. J. Med. Microbiol. 60: 983-712.
Shah AA, Hasan F, Ahmed S, Abdul Hameed (2004). Characteristics, epidemiology and clinical importance of emerging strains of Gram-negative bacilli producing extended-spectrum β-lactamases. Research in Microbiology.155:409–421.
Touati A, Benallaoua S, Forte D, Madoux J, Brasme L, de Champs C (2006). First report of CTX-M-15 and CTX-M-3 beta-lactamases among clinical isolates of *Enterobacteriaceae* in Béjaia, Algeria. Int. J. Antimicrob. Agents. 27: 397-402.
Zaoutis TE, Goyal M, Chu JH, Coffin SE, Bell LM, Nachamkin I, McGowan KL, Bilker WB, Lautenbach E (2005). Risk factors for and outcomes of bloodstream infection caused by extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella* species in children. Pediatrics. 4: 942-949.