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Characterization of extended-spectrum β-lactamase (ESBL)-producing *Klebsiella, Enterobacter*, and *Citrobacter* obtained in environmental samples of a Tunisian hospital

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ABSTRACT

The assessment of the hospital environment as a reservoir of ESBL-producing *Enterobacteriaceae* in Tunisian hospitals is scarcely analyzed, except for *Escherichia coli.* The aim of this study was to evaluate the presence of ESBL-producing non-*E. coli Enterobacteriaceae* (ESBL-EbNoEc) in 300 samples of abiotic surfaces and the hands of patients and staff of a Tunisian Hospital, and to characterize the ESBL genes of the recovered isolates. ESBL-EbNoEc were recovered in 28 of 300 (9.3%) analyzed samples and were identified as *Klebsiella pneumoniae* (n= 11), *Enterobacter cloacae* (n=11), *Citrobacter freundii* (n=4) and *Klebsiella oxytoca* (n=2). The *bla* genes identified by PCR and sequencing among the strains were as follows: 11 *K. pneumoniae* strains [$bla_{CTX\text{-}M\text{-}15}$+ $bla_{TEM\text{-}1}$+ $bla_{SHV\text{-}11}$ (n=6); $bla_{CTX\text{-}M\text{-}15}$+ $bla_{TEM\text{-}1}$+ $bla_{SHV\text{-}28}$ (n=3); $bla_{CTX\text{-}M\text{-}15}$+ $bla_{TEM\text{-}1}$+ $bla_{SHV\text{-}1}$ (n=2)], 11 *E. cloacae* strains [$bla_{CTX\text{-}M\text{-}15}$ (n=6); $bla_{CTX\text{-}M\text{-}15}$+ $bla_{TEM\text{-}1b}$ (n=2); $bla_{CTX\text{-}M\text{-}15}$+ $bla_{TEM\text{-}1b}$+ $bla_{OXA\text{-}1}$ (n=1);$bla_{CTX\text{-}M\text{-}15}$+ $bla_{OXA\text{-}1}$ (n=1);$bla_{SHV\text{-}12}$ (n=1)], 4 *C. freundii* strains [$bla_{CTX\text{-}M\text{-}15}$] and 2 *K. oxytoca* strains [$bla_{CTX\text{-}M\text{-}15}$ (n=1); $bla_{SHV\text{-}12}$ (n=1)]. The IS*Ecp1* and *orf477* sequences were identified upstream and downstream of the $bla_{CTX\text{-}M\text{-}15}$ gene, respectively, in 3 *K. pneumoniae* and 3 *E. cloacae* isolates. The PFGE analysis demonstrated three unrelated pulsotypes in *K. pneumoniae* strains and five pulsotypes in *E. cloacae*. The uncontrolled dissemination of ESBL-producing bacteria, even in the hospital environment, has become a real problem and new strategies and hygienic rules are needed to stop this bacterial dissemination.

1. Introduction

Inanimate objects and surfaces in the surroundings of patients can be easily contaminated with pathogenic or antimicrobial-resistant bacteria, posing a risk factor for the transmission of these microorganisms to other patients or staff (Guet-Revillet et al., 2012). Actually, these pathogenic bacteria have an innate ability to survive on dry surfaces in the hospital environment for several days (Hota, 2004). However, the role of these abiotic surfaces in the transmission of ESBL-producing *Enterobacteriaceae* has not been given the priority it deserves.

The treatment of infections caused by these bacteria is complicated because of their frequent resistance to several families of antibiotics (Gholipour et al., 2014). Genes encoding ESBLs are usually carried on plasmids, which facilitate their spread among various Gram-negative bacteria species (Silva-Sanchez et al., 2011). *Klebsiella pneumoniae* and *Enterobacter cloacae* are important causes of nosocomial infections and were reported to present increasing resistance to many antimicrobials (Souna et al., 2014; Meatherall et al., 2009; Yang et al., 2014). A previous study performed in our group showed that ESBL-producing *E. coli* was detected in 4% of abiotic surfaces tested from a Tunisian Hospital (Dziri et al., 2016); nevertheless, few data exist about the contamination level by ESBL-producers of other members of the *Enterobacteriaceae* familly. For this reason, the aim of this study was to detect and characterize ESBL-producing *Enterobacteriaceae* strains others than *E. coli* (ESBL-EbNoEc), isolated from abiotic surfaces and the hands of patients and staff in the Tunisian Hospital and to determine the clonal diversity of recovered isolates by pulsed-field gel electrophoresis (PFGE). This study will provide more information about the reality of ESBL prevalence in the hospital environment and will represent a valuable help to control this emerging problem.

2. Material and methods

2.1. Samples and bacterial isolates

Three hundred samples were taken from abiotic surfaces (beds, treatment tables, toilets, faucets, Handle doors, sinks), and from the

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hands of patients and sanitary staff in 17 different services (Neonatology, Gynecology, Urology, Gastrology, Internal Medicine, Dermatology, Pediatric, Cardiology, Hemodialysis, Orthopedic Surgery, Ophthalmology, Trauma, Endocrinology, Pneumonology, Emergency, Intensive Care Unit, Visceral and General Surgery) of the Military Hospital of Tunis during March to June 2013. Samples were inoculated on MacConkey agar plates and incubated for 24 h at 37 °C. One suspected colony of *Enterobacteriaceae* non-*E. coli* (EbNoEc) per positive plate was selected and identified by molecular tests (PCR and sequencing of 16S rDNA).

2.2. Antibiotic susceptibility testing

The detection of ESBL producing strains was performed by the double disk diffusion test using cefotaxime, ceftazidime and amoxicillin/clavulanic acid disks. Isolates were tested for susceptibility to the following 15 antibiotics (in μg/disk): ampicillin (10), amoxicillin/clavulanic acid (20/10), ticarcillin (75), cefoxitin (30), ceftazidime (30), cefotaxime (30), imipenem (10), gentamicin (10), tobramycin (10), streptomycin (10), nalixidic acid (30), ciprofloxacin (5), trimethoprim/sulfamethoxazole (1.25/23.75), tetracycline (30) and chloramphenicol (30). The results were interpreted according to the Clinical and Laboratory Standards Institute criteria (CLSI, 2016).

2.3. Characterization of beta-lactamase genes and genetic environment of $bla_{CTX\text{-}M}$genes

The genes encoding CTX-M, TEM, SHV and OXA type beta-lactamases were analyzed by PCR and sequencing in all ESBL isolates (Jouini et al., 2007; Rocha-Gracia et al., 2010). The presence of IS*Ecp1* and *orf477* sequences surrounding the $bla_{CTX\text{-}M}$ genes were analyzed by PCR and sequencing using primers and conditions, as previously described (Eckert et al., 2006).

2.4. Genetic typing of isolates

The clonal relationship among our strains was determined by pulsed field gel electrophoresis (PFGE) using *XbaI* enzyme, as previously described (Sáenz et al., 2004).

3. Results

3.1. Prevalence of ESBL-EbNoEc in the hospital environment

ESBL-EbNoEc were recovered in 28 of 300 (9.3%) analyzed hospital environmental samples. These isolates were recovered from 25 of 250 abiotic surfaces (10%) and from 3 of 50 hands of patients/staff (6%) and were obtained in 9 (Urology, Gyneocology, Ophthalmology, Dermatology, Pneumonology, Neonatology, Cardiology, Gastrology, Orthopedic Surgery) of the 17 hospital services tested. They were identified as (number of isolates and number from abiotic surfaces/human hands): *K. pneumoniae* (n=11, 10/1), *E. cloacae* (n=11, 11/0), *Citrobacter freundii* (n=4, 3/1) and *Klebsiella oxytoca* (n=2, 1/1). The majority of *K. pneumoniae* and *E. cloacae* isolates were recovered from the Urology Service and most of *C. freundii* isolates from Gyneocology Service (Table 1).

3.2. Molecular characterization of genes encoding ESBL and their genetic environment

The *bla* genes identified among the ESBL-positive isolates of different species were as follows (Tables 1 and 2): 11 *K. pneumoniae* strains [$bla_{CTX\text{-}M\text{-}15}$+ $bla_{TEM\text{-}1}$+ $bla_{SHV\text{-}11}$ (n=6); $bla_{CTX\text{-}M\text{-}15}$+ $bla_{TEM\text{-}1}$+ $bla_{SHV\text{-}28}$ (n=3); $bla_{CTX\text{-}M\text{-}15}$+ $bla_{TEM\text{-}1}$+ $bla_{SHV\text{-}1}$ (n=2)], 11 *E. cloacae* strains [$bla_{CTX\text{-}M\text{-}15}$ (n=6); $bla_{CTX\text{-}M\text{-}15}$+ $bla_{TEM\text{-}1b}$ (n=2); $bla_{CTX\text{-}M\text{-}15}$+ $bla_{TEM\text{-}1b}$+ $bla_{OXA\text{-}1}$ (n=1); $bla_{CTX\text{-}M\text{-}15}$+ $bla_{OXA\text{-}1}$ (n=1); $bla_{SHV\text{-}12}$ (n=1)], 4 *C. freundii* strains [$bla_{CTX\text{-}M\text{-}15}$] and 2 *K. oxytoca* strains [$bla_{CTX\text{-}M\text{-}15}$ (n=1); $bla_{SHV\text{-}12}$ (n=1).

The IS*Ecp1* sequence was detected in 9 *K. pneumoniae* and 4 *E. cloacae* isolates, and the *orf477* in 4 *K. pneumoniae*, 8 *E. cloacae* and 3 *C. freundii* isolates. The IS*Ecp1* and *orf477* sequences were identified upstream and downstream, respectively, of the $bla_{CTX\text{-}M\text{-}15}$ gene in 3 *K. pneumoniae* and 3 *E. cloacae* isolates.

3.3. Characterization and genetic relatedness of K. pneumoniae and E. cloacaeisolates

Tables 1 and 2 show the characteristics of *K. pneumoniae* and *E. cloacae* isolates respectively. The majority of *K. pneumoniae* isolates were obtained from the Urology service and all of them harbored the genes $bla_{CTX\text{-}M\text{-}15}$, and $bla_{TEM\text{-}1,}$ as well as different variants of bla_{SHV} ($bla_{SHV\text{-}1,}$$bla_{SHV\text{-}11,}$ and $bla_{SHV\text{-}28}$). The PFGE analysis of these 11 *K. pneumoniae* isolates demonstrated three unrelated pulsotypes, and each pulsotype showed a close association with the origin of the sample (Table 1).

Similar results were found for *E. cloacae* isolates, where most of them were also from the Urology service, and all except one, harbored the $bla_{CTX\text{-}M\text{-}15}$ gene. Five pulsotypes were identified among *E. cloacae* isolates, with dominance of pulsotype E3 (Table 2).

4. Discussion

Our study shows that bacteria belonging to the *Enterobacteriaceae* family of the genera *Klebsiella, Enterobacter* and *Citrobacter* are able to survive both on abiotic surfaces and on the hands of persons who may transmit pathogens from one surface to another, or even from the environmental area to patients and staff. Our data demonstrate that *K. pneumoniae* and *E. cloacae* were the predominant species among ESBL-EbNoEc, which were isolated in great number of samples from the Urology service. The cleaning practices of this area could have an

Table 1
Characterization of the 11 *K. pneumoniae* strains isolated from environmental samples of a Tunisian Hospital.

Strain	Origin	Service	Phenotype of resistance for non-beta-lactams[a]	Beta lactamases	Genetic environment of $bla_{CTX\text{-}M}$ gene	Pulsotype (PFGE)
C7124	Treatment table	Cardiology	TE, C, SXT, NA, TM	CTX-M-15, TEM-1b, SHV-28	IS*Ecp1*-*bla*-Orf477	K1
C7125	Cardioline	Cardiology	TE, C, SXT, NA, TM	CTX-M-15, TEM-1b, SHV-28	IS*Ecp1*-*bla*-Orf477	K1
C7126	Sink	Cardiology	TE, C, SXT, NA, TM	CTX-M-15, TEM-1b, SHV-28	IS*Ecp1*-*bla*-Orf477	K1
C7120	Handle door	Urology	TE, GM, C, SXT, TM	CTX-M-15, TEM-1b, SHV-11	IS*Ecp1*-*bla*	K2
C7121	Handle door	Urology	TE[I], SXT	CTX-M-15, TEM-1b, SHV-11	IS*Ecp1*-*bla*	K2
C7122	Table	Urology	TE[I], SXT	CTX-M-15, TEM-1b, SHV-11	IS*Ecp1*-*bla*	K2
C7134	Hand of patient	Urology	TE, SXT, NA	CTX-M-15, TEM-1b, SHV-11	IS*Ecp1*-*bla*	K2
C7135	Bedlinen	Urology	SXT, TM	CTX-M-15, TEM-1b, SHV-11	IS*Ecp1*-*bla*	K2
C7284	Table	Gastrology	SXT,	CTX-M-15, TEM-1b, SHV-11	IS*Ecp1*-*bla*	K2
C7127	Toilet	Gynecology	TE, C, SXT, NA, TM	CTX-M-15, TEM-1b, SHV-1	*bla*	K3
C7118	Bedlinen	Neonatology	TE, GM, SXT, NA, TM	CTX-M-15, TEM-1a, SHV-1	*bla*-Orf477	NT[b]

[a] TE = tetracycline; GM = gentamicin; C = chloramphenicol; SXT = trimethoprim-sulphametoxazole; NA = nalidixic acid; TM = tobramycin.
[b] NT: non typeable (non clear results by PFGE).