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Distribution of extended-spectrum beta-lactamase TEM and CTX-M resistance genes among *Proteus* species isolated in Sudan

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Proteus species are found in the human intestinal tract as part of normal flora. *Proteus* species are also found in multiple environmental habitats, including long-term care facilities and hospitals, and can cause both community and nosocomial infections. For a long time *Proteus* was known to be susceptible to beta-lactam antibiotics but nowadays they become resistant. The aim of this study was to detect the Extended-spectrum beta-lactamase (ESBL) TEM and CTX-M genes in 90 *Proteus* species isolated from urine and wound swabs, obtained from different hospitals in Khartoum state, Sudan, from January to August 2018. Antimicrobial sensitivity was carried out using the following set of antibiotics: amoxiclav, ceftazidime, gentamicin, meropenem, cefotaxime, ciprofloxacin, amoxicillin, ceftriaxone and cotrimoxazole. ESBL producing strains were detected by double disc diffusion synergy test and the resistance genes TEM and CTX-M were detected by Polymerase Chain Reaction (PCR). Antibiotic resistance was found: amoxicillin 40%, ceftazidime 25.6%, ceftriaxone 23.3%, gentamicin 22.2%, cotrimoxazole 21.1%, and cefotaxime 18.9%. Most of the isolates were sensitive to meropenem 92.2% and ciprofloxacin 86.7%. In double-disk diffusion synergy test, 20 isolates (22.2%) were found to be positive for ESBL. The PCR demonstrated that TEM gene was present in 18 isolates (90%). It was present alone in 11 isolates (55%) and in combination with CTX-M gene in seven isolates (35%). The percentage of ESBL producing strains of *Proteus* was 23.5%. This percentage is a bit lower than in previous studies in Sudan. In conclusion; it seems that the CTX-M gene is emerging among *Proteus* species in Sudan.

Keywords: Sudan; *Proteus*; beta-lactamase; genes; nosocomial infections.

Introduction

Infectious diseases continue to be a major cause of morbidity and mortality.(1) Multidrug-resistant members of the family of *Enterobacteriaceae* are responsible for such infections.(2)

Patients with infections due to extended-spectrum beta-lactamase (ESBL) producing organisms are likely to have a poor outcome when compared to those infected with non-producing organisms.(3)

ESBL are enzymes produced by many gram-negative bacteria. They have the ability to inactivate the third generation cephalosporin, penicillin, and the monobactam antibiotics. However, they are inhibited by clavulanic acid.(4,5)

Several investigations have reported a different prevalence of ESBLs ranging from 6% to 88% in various health care setting especially among members of *Enterobacteriaceae*. Although TEM, SHV genes were the most common ESBL producing genes.(6,7) Most of the gram-positive bacteria produce their beta-lactamases in the surrounding, thus inactivating beta-lactam antibiotics externally. And by contrast, the beta-lactamases of the gram-negative bacteria remain inside the cells inactivating the drug in the periplasmic space.(8)

Proteus species are members of the family *Enterobacteriaceae*, present as normal flora of the human intestine and in various environmental habitats including hospitals. They can cause both community and nosocomial acquired infections.(9)

For a long time, *Proteus* was known to be susceptible to beta-lactam antibiotics. Nowadays they are becoming resistant due to the spread of extended-spectrum beta-lactamase.(10) The aim of this study was to detect the beta-lactamase TEM and CTX-M genes in clinical isolates of *Proteus* species from different hospitals in Khartoum state, Sudan.

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Materials and Methods

This prospective hospital-based cross-sectional study was carried out from January to August 2018. Ethical considerations of the research objectives were approved by the National Ribat University ethical committee. Voluntary informed consent form was signed by each participant after explaining the objectives of the study. Ninety *Proteus* strains were isolated from urine and wound swabs, the isolation and identification of pure culture were achieved based on colonial morphology, gram stain and set of biochemical tests.

Isolates were selected on the following profile of identification: gram negative bacilli, non-lactose fermenter and urease positive.

Antimicrobial sensitivity was carried out according to the CLSI method using the following set of antibiotics: amoxiclav (AMC), ceftazidime (CAZ), gentamicin (GN), meropenem (MEM), cefotaxime (CFM), ciprofloxacin (CIP), amoxicillin (AX), ceftriaxone (CRO) and cotrimoxazole (STX).[11]

ESBL producers were detected by a double disc diffusion synergy test.[11] The TEM and CTX-M genes were detected by the DNA guanidine chloride extraction.[12] The Polymerase Chain Reaction (PCR) was done using primers for the TEM and CTX-M genes (Table 1).

The reaction mix and procedure protocol and optimization were applied according to product instruction sheet (Promega Corporation, USA). The results of antibiotics susceptibility were calculated based on the percentage of the number of isolates that show sensitivity or resistance toward each antibiotic divided by the total number of isolates (90).

Table 1. The primer sequences for the TEM and CTX-M genes.

ESBL Gene	Primer's sequence (nucleotides)
TEM-forward	5'- ATGAGTATTCAACATTTCCGTG- 3'
TEM-reverse	5'- TTACCAATGCTTAATCAGTGAG- 3'
CTX-M-forward	5'- GGTTAAAAAATCACTGCGTC- 3'
CTX-M-reverse	5'- TTACAAACCGTCGGTGACGA- 3'

Results and Discussion

The results of antibiotic sensitivity showed that most of the isolated strains were sensitive to meropenem: 83 (92.2%) and ciprofloxacin: 78 (86.7%) (Fig. 1). While the highest antibiotic resistance were obtained with amoxicillin: 36 (40%), ceftazidime: 23 (25.6%), ceftriaxone: 21 (23.3%), gentamicin: 20 (22.2%), cotrimoxazole: 19 (21.1%), and cefotaxime: 17 (18.9%) (Fig. 2).

Twenty isolates (22.2%) were found to be positive for ESBL by double-disk diffusion synergy test, then ESBL resistant genes were detected by PCR using selected primers. TEM and CTX-M genes were present in 18 isolates (90%). TEM was present alone in 11 isolates (55%) and CTX-M gene was present in seven isolates (35%) (Fig. 3).

None of the CTX-M genes were detected separately. While two isolates out of the 20 ESBL producers were negative for both TEM and CTXM genes (10%). The common pattern of resistance associated with both the TEM and CTX-M gene carriers was a combination of ceftazidime, ceftriaxone and amoxicillin (83.3%).

The presence of ESBL producing bacteria is striking rapid worldwide, hence the increase of resistance to antibiotics and the emergence of multidrug-resistant ESPL producers are becoming a public health problem, causing clinical failure of empirical antibiotic treatment.[13] Consequently, a continuous monitoring system and effective control measures are absolutely required.

The percentage of ESBL *Proteus* strains in this study was 22.2%. This percentage is a bit lower than in previous studies in Sudan, where the detected percentage was 29.6% in 2016 and 33.3% in 2013.[14,15] It was even lower in comparison with a study in Turkey, where the percentage of ESPL producers were 48.5%.[6]

Plasmids with Multidrug-resistant genes are common among the family of *Enterobacteriaceae*. Historically, *Proteus* species were known to be free of the beta-lactamase genes.[16] However, *Proteus*, as a member of the family *Enterobacteriaceae*, can acquire the plasmids from other members of the family.

For a long time, *Proteus* species are known to carry the TEM beta-lactamase gene only.[17] Nowadays, the currently spreading beta-lactamase is the CTX-M gene. Generally, the CTX-M gene is replacing the TEM gene and the SHV genes.

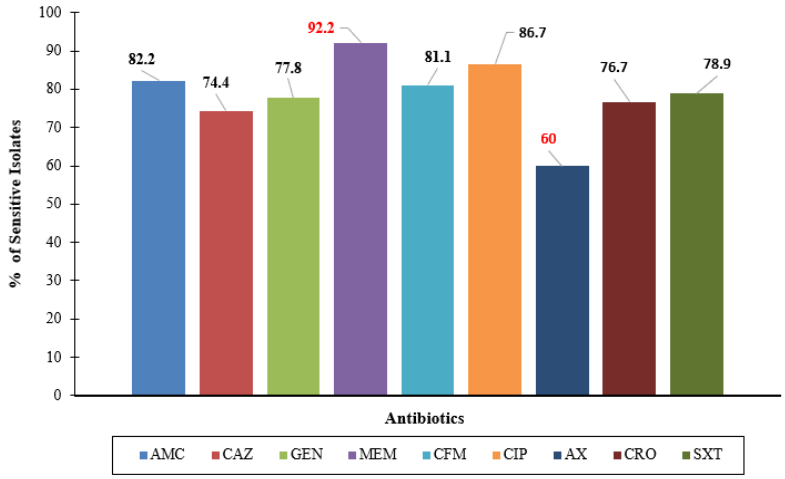

Fig. 1. Antibiotic sensitivity (%) among *Proteus* strains isolated in Sudan: amoxiclav (AMC), ceftazidime (CAZ), gentamicin (GN), meropenem (MEM), cefotaxime (CFM), ciprofloxacin (CIP), amoxicillin (AX), ceftriaxone (CRO) and cotrimoxazole (SXT).

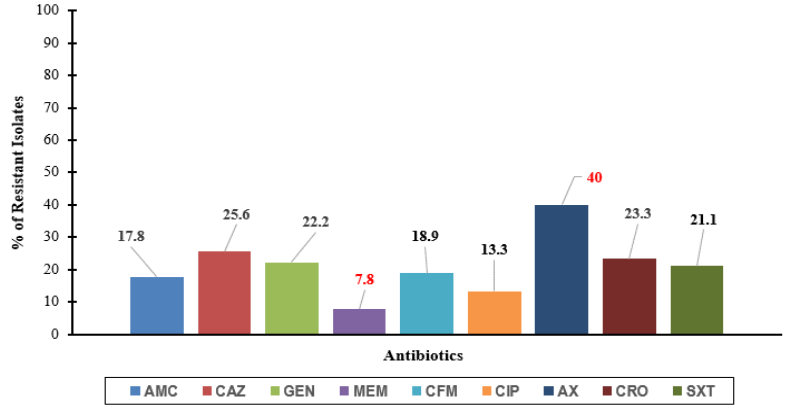

Fig. 2. Antibiotic resistance (%) among *Proteus* strains isolated in Sudan: amoxiclav (AMC), ceftazidime (CAZ), gentamicin (GN), meropenem (MEM), cefotaxime (CFM), ciprofloxacin (CIP), amoxicillin (AX), ceftriaxone (CRO) and cotrimoxazole (SXT).

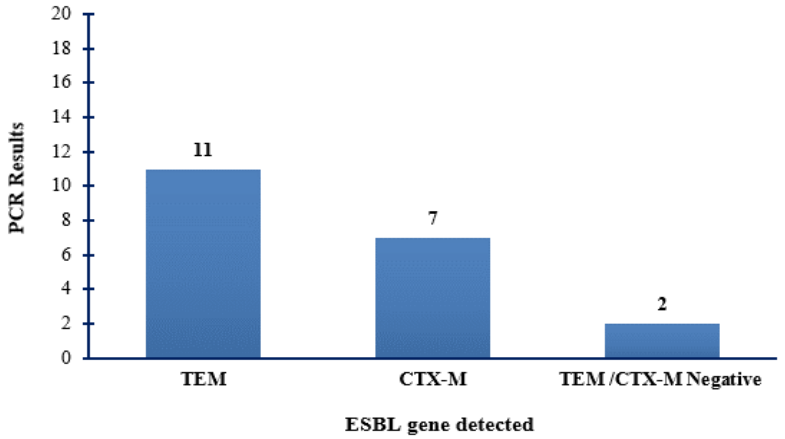

Fig. 3. Extended-spectrum beta-lactamase producers TEM and CTX-M genes detected by PCR out of 20 ESBL positive samples by double-disk diffusion synergy test.

In this study, the TEM gene was detected alone in 55% of the ESBL producers and in 35% in combination with the CTX-M gene. It seems that the CTX-M gene is emerging among *Proteus* species. In various studies conducted in India, *Proteus* species were carrying the TEM gene only.[18,19] In Italy, 44% of the ESBL producing *Proteus* were also carrying the TEM gene only.[20] While in Iraq, all ESBL producing *Proteus* had this gene.[21] In recent studies performed in India, 35.3% of the ESBL producing *Proteus* were carrying CTX-M gene alone; the 1.8% of strains the TEM gene and the 52.9% both genes together.[22]

The CTX-M gene appears to emerge in combination with TEM gene at the beginning and then replacing the others as the dominant gene is spreading. It seems that the selective pressure by the misuse of antibiotics has created a favorable environment for the spread of ESBLs among *Enterobacteriaceae*.

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Conflicts of interest

The authors declare there are no conflicts of interest.

Distribución de genes de resistencia de betalactamasas de espectro extendido TEM y CTX-M entre especies de *Proteus* aisladas en Sudán

Resumen

Las especies de *Proteus* se encuentran en el tracto intestinal humano y forman parte de su flora normal. También se localizan en el medio ambiente y otros hábitats, incluyendo hospitales y diversas instituciones de salud, provocando tanto infecciones en la comunidad como nosocomiales. Durante mucho tiempo, las especies de *Proteus* fueron susceptibles a los antibióticos betalactámicos, pero actualmente se han tornado resistentes. El propósito de este estudio fue detectar genes de resistencia betalactamasas de espectro extendido (BLEE) TEM y CTX-M, en 90 especies de *Proteus* aisladas en orina y heridas, provenientes de diversos hospitales del estado de Jartum, Sudán, entre enero y agosto de 2018. La sensibilidad antimicrobiana se determinó con el siguiente juego de antibióticos: amoxiclav, ceftazidima, gentamicina, meropenem, cefotaxima, ciprofloxacina, amoxicilina, ceftriaxona y cotrimoxasol. Las cepas productoras de BLEE se detectaron mediante la técnica de sinergia de doble disco, y los genes de resistencia TEM y CTX-M mediante Reacción en Cadena de la Polimerasa (PCR). Se encontró resistencia antibiótica: amoxicilina 40%, ceftazidima 25,6%, ceftriaxona 23,3%, gentamicina 22,2%, cotrimoxasol 21,1% y cefotaxima 18,9%. La mayor parte de los aislamientos fueron sensibles a meropenem (92,2%) y ciprofloxacina (86,7%). Con la técnica de sinergia de doble disco se detectó positividad a BLEE en 20 aislamientos (22,2%). Mediante PCR se demostró que el gen que codifica TEM estaba presente en 18 aislamientos (90%); de forma aislada en 11 aislamientos (55%) y combinado con el gen CTX-M en los otros siete (35%). El porcentaje de cepas de *Proteus* productoras de BLEE fue de 23,5%. Este valor es ligeramente inferior que los detectados en estudios previos en Sudán. En conclusión, hay evidencias de que el gen CTX-M está emergiendo entre las especies de *Proteus* en Sudán.

Keywords: Sudan; *Proteus*; betalactamasa; genes; infecciones nosocomiales.

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