https://doi.org/10.46389/rjd-2024-618
www.jrdiabet.ro
Rom J Diabetes Nutr Metab Dis
2024; Volume 31, Issue 1, pages 618-624

THE DETECTION OF *OMPX* GENE AND ANTIBIOTIC RESISTANCE OF *ENTEROBACTER CLOACAE* PRODUCED BIOFILM ISOLATED FROM CLINICAL SOURCES IN SOME HOSPITALS ANBAR PROVINCE

Esraa sufyan nohad[1], Prof. Dr. Muthanna Hamid Hassan[2]
[1,2]Department of Biology, College of Science, University of Anbar, Iraq
Sc.muthanna-477-aliraqi@uoanbar.edu.iq

Abstract
The study included the isolation and identification of E. cloacae from clinical sources. The results showed that fifteen isolates from a total of 150 different samples were identified as *E. cloacae* by morphological, microscopic and biochemical tests and were confirmed with the Vitek II system. These results showed a close relationship between the presence of the *OmpX* gene and its multi-drug resistance and ability to produce biofilm. Accordingly, all clinical isolates can be classified as multi-drug resistant. These results indicate the possibility of creating multi-drug-resistant Enterobacteriaceae for clinically isolated bacteria. The results showed that fourteen isolates produced strong biofilms and only one had weak biofilms.

Keywords: *Enterobacter cloacae* complex,OmpX gene,Biofilm,Antibiotic Resistance

Introduction

Bacteria belonging to Enterobacter cloacae complex (ECC) are opportunistic Gram-negative bacilli that comprise a part of the healthy gut microbiota of humans and animals. ECC are also members of the ESKAPE group of pathogens and are frequently associated with MDR nosocomial infections (1). The infections caused by *E. cloacae* are most frequently brought on by healthcare-associated pathogens in immunocompromised patients, such as the elderly and those suffering from many medical conditions. *E. cloacae* has also emerged as a serious nosocomial pathogen in neonatal intensive care units (2).Numerous innate and acquired resistance mechanisms to antibiotics necessary for treating human disease have been discovered in this organism, and selective factors push *E. cloacae* toward a multidrug-resistant phenotype (3). The *E. cloacae* complex has evolved resistance to several different types of antibiotics, and this class of antibiotics is just one of them. These antibiotics operate on bacteria by interacting with type II topoisomerases (DNA gyrase and topoisomerase IV). The bacteria have evolved resistance mechanisms that either result in reduced access to the target itself by either decreasing permeability or increasing expression of efflux pumps, such as AcrAB and MexAB, or in target mutations, such as GyrA/GyrB for DNA gyrase and ParC/ParE for topoisomerase IV(4,5). The OmpX inhibits the host's defensive mechanisms establishes a physical connection between the outer membrane and the peptidoglycan layer. While the expression of some porins, such as OmpA, remains constant in cells, other porins, including LamB, PhoE, and FhuA, are triggered by either the presence of a particular substrate or external stimuli(6). The aim of this research is study a correlation between *OmpX gene* ,antibiotic resistance and biofilm produced in ECC clinical isolates hospitals in Anbar Governorate.

Methodology

in this study have collection about 150 samples from patients suffering from urinary tract infections, burns and wounds. The ages included in the study were between (15-64) years old for both sexes. The period of collection of samples extended from April 2022 to January 2023. collected from Al-Fallujah and Al Ramadi Teaching Hospitals from hospitalized patients. Smear from infectious area was taken by sterilized cotton swab, while urine samples from mid stream urine in clean container and then samples had been inoculated on the culture media (MacConkey, chocolate and Blood agar) and incubated aerobically at 37°C for 24 hrs.

After the positive culture appeared. Then reward of Microscopic identification by using gram stain:. Biochemical Identification included :Indole test, Methyl red test,Voges –Proskauer test and Citrate utilization test (7). The diagnosis was confirmed by using the Vitek II system(8).

Antibiotic susceptibility:

Susceptibility test was performed by the automated Vitek-2 system used an AST card and also by used the disc diffusion method (Kirby-Bauer technique).

Molecular study:

Extraction of DNA: The FavorPrepTM / Cultured Cell Genomic DNA Extraction Mini Kit (Taiwan) using to extract genomic DNA from *Enterobacter cloacae* Complex isolates .

Detection of genes:

The thermocycler (Bioapplied cycler®, USA) was used to create and amplify the PCR reactions.

Detection of OmpX gene: The PCR program for detection of OmpX was initial denaturation 95°C for 3 min. 40 cycles of denaturation 95°C for 30 s, annealing 60°C for 45 s , extension 72°C for 60 s with a final elongation step at 72°C for 7 min.

Table 1:Reaction components of genes.

Component	25µL (Final volume)
Taq PCR PreMix	5µl
Forward primer	10 picomols/µl (1 µl)
Reverse primer	10 picomols/µl (1 µl)
DNA	1.5µl
Distill water	16.5 µl

Following PCR amplification was confirmed using agarose gel electrophoresis. The criteria based on the extracted DNA were completely reliable for PCR.

Biofilm formation assay

The procedure that was adopted in this study was performed according to [9] by the wells of sterile 96 well U shaped –bottomed polystyrene microplates .

Results and Discussion

Isolation:

About only fifteen (12.5%) of the positive culture samples were identified as *Enterobacter* and the remaining 105/120 (87.5%) contained isolates from other bacterial genera, as shown in Figure (1). While 30 samples were originally negative for microbial culture

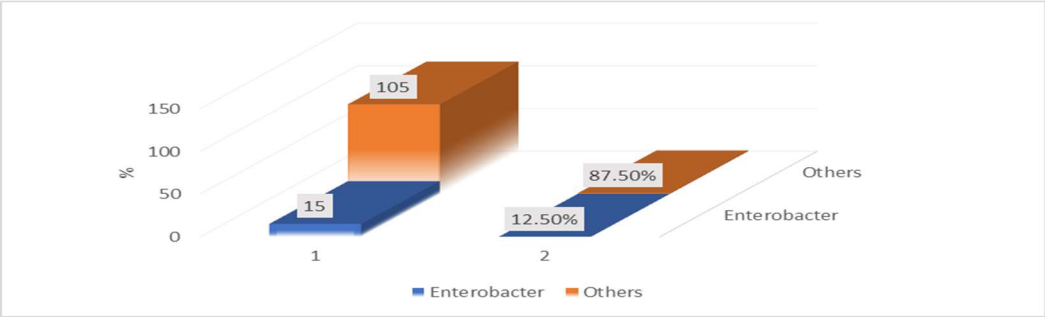

Figure 1 : Bacterial Isolates recovered from the Samples.

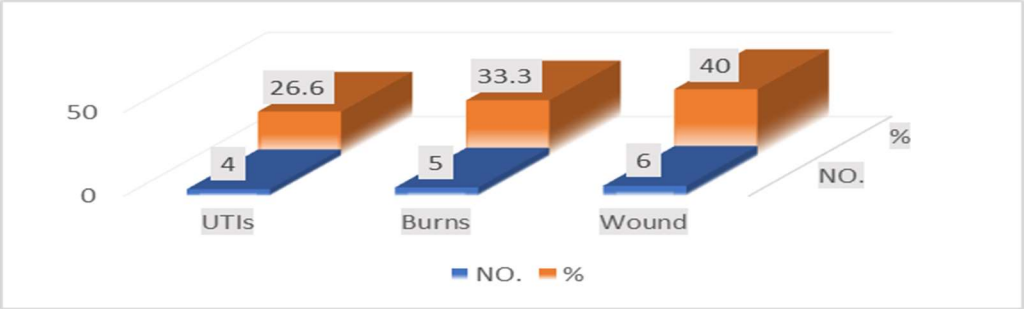

Figure 2 : Distribution of isolated bacteria according to the source.

According to Figure (2), swabs from wound infection showed a higher percent of Enterobacter isolates 6/15(40%), while the UTI samples showed less distribution of bacteria 4/15(26.6%).

To confirm the initial diagnosis, at first, *Enterobacter* were characterized by morphological, microscopical in addition to being cultivated on MacConkey agar and Blood agar under aerobic conditions. When grown on the MacConkey agar medium, results showed that the colonies appear as pink to red, lactose-fermenting, slightly mucoid colonies similar in appearance to *Klebsiella pneumoniae* as illustrated. On Blood agar media they grow as non hemolytic grey-white colonies after 24 hrs of incubation.(10). Also several biochemical tests were done for further identification and characterization of *Enterobacter,* the results of biochemical tests and result of Gram stain were summarized in the Table 2.

Table 2:Biochemical tests for characterization of *Enterobacter cloacae* complex:

Enterobacter	Indole	Methyl red	VP	Citrate	Urase
	-	-	+	+	+

(+) positive result, (-) negative result, (VP) Voges –Proskauer test.

Fifteen isolates showed negative for indole, positive for citrate, positive for vp, and negative for methyl red and gram stain.

Automated Identification by Using Vitek II System:

Enterobacter cloacae complex (ECC) was identified successfully by using the VITEK 2 compact System GN cards (BioMerieux France).(11) .

Test for Antibiotic Susceptibility:

The Vitek 2 Technology was used to verify the antibiotic sensitivity of all Fifteen *Enterobacter cloacae* complex (ECC) samples from patients with wound, burn, and urinary tract infections.

Susceptibility was examined to seventeen antimicrobials including: Piperacillin/Tazobactam, Cefazolin, Cefoxitin, Ceftazidime, Ceftriaxone, Cefepime, Ertapenem, Imipenem, Gentamicin, Ciprofloxacin, Levofloxacin, Tigecycline, Nitrofurantoin, Trimethoprim/ Sulfamethoxazole, Meropenem, Amoxicillin/clavulanic acid, and norfloxacin. The results of antimicrobial susceptibility test rates of the fifteenth ECC isolates revealed a various resistance levels to antimicrobial agents as following: Piperacillin/Tazobactam 40%, Cefazolin 100%, Cefoxitin100%, Ceftazidime 100%,Ceftriaxone 100%, Cefepime 100%, Ertapenem 6.66%, Imipenem 40%, Gentamicin 40%, Ciprofloxacin 33.3%, Levofloxacin 33.3%, Tigecycline 0%, Nitrofurantoin0%, Trimethoprim/ Sulfamethoxazole 66.6%, Meropenem 100%, Amoxicillin/clavulanic acid 100% , and norfloxacin 33.3% as showed in Figure 3
The above findings showed that the isolates were resistant to every antibiotic

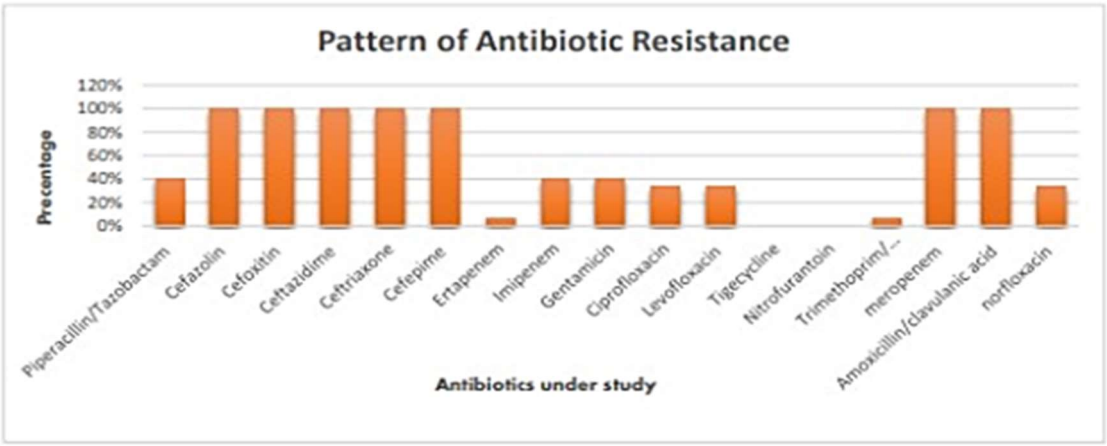

Figure 3:Pattern of Antiibiotic Resistance in Enterobacter cloacae complex

The current study showed that ECC possess the highest resistance (100%) toward the Cephalosporins class, which includes several generations like Cefazolin,Cefoxitin, Ceftazidime, Ceftriaxone and Cefepime, Fifty strains were resistant to ceftazidime, while 54 were resistant to ceftriaxone , according to a study conducted in Twain. There were three groups of 50 ceftazidime-resistant isolates: One was susceptible, two were intermediately susceptible, and 47 were resistant to ceftriaxone. According to reports, third-generation cephalosporins were resistant to more than 70% of *Enterobacter* isolates in Poland; similarly, data from France and Guadeloupe showed that *E. hormaechei* had greater rates of resistance to third-generation cephalosporins when compared to other *Enterobacte*r clusters (12). In contrast, our results revealed that the resistance rate for Amoxicillin/clavulanic acid was 100%. Augmentin belongs to the penicillin-like antibiotics class of medicines. It works by preventing bacteria from growing.
In Figure (3), ECC was 40% resistant to piperacillin/tazobactam.and to toward
Meropenem was 100% resistant, ECC was resistant to Imipenem at 40%, and less resistant to Ertapenem was 6.66%. These are intravenous-lactam antibiotics from the Carbapenems class, Carbapenem-resistant Enterobacteriaceae (CRE) have emerged as a significant global public health concern, and E. cloacae complex that was carbapenem-resistant was concentrated in the

Southwest and Pacific Coast by 2014-2015. It is challenging to choose an effective treatment for Enterobacter spp.because they have an inherent resistance to ampicillin and broad-spectrum cephalosporins and have acquired genetic mobile elements that make them resistant to many antibiotics, including third-generation cephalosporins and carbapenems(13).

The result reveled that the resistance of ECC isolates toward Quinolones class of antibiotic which include Ciprofloxacin , Levofloxacin and Norfloxacin were the same in percentage 33.3%, while no resistance or low MICs against ciprofloxacin and Levofloxacin found in a study conducted by (14).

In Figure 3, Resistance of ECC isolates to Gentamicin was (40%). A similar tendency was observed in a prior study where their ECC clinical isolates showed approach results to Gentamicin by percentages (50%) (15). The current study also demonstrated that ECC resistance to Trimethoprim/ Sulfamethoxazole was 66.6%. Similar findings were observed in Iran, where clinical ECC resistance to Trimethoprim/ Sulfamethoxazole was 60.4% (15).It is worth mentioning that in this study all our ECC isolates were susceptible to the Tigecycline and Nitrofurantoin , which can be consider the most effective agents against ECC under study. When treating ECC infection, antibiotics should be given in a systematic, nonrandom manner. Multi-drug resistant (MDR) ECC strains, which constitute for 65 to 75 percent of Enterobacter infections, have emerged and spread as a result of antibiotic use (16).

Detection of OmpX gene by conventional PCR techniques:

By employing a particular primer that focuses on the precise sequence of the target gene, the OmpX gene was detected using the conventional PCR approach for all of the isolates currently under study. Then, 1.5% agarose gel was used to place the amplified products For 1.5 hours afterwards. Results showed that 15/15 (100%) of the isolates had this gene, and the bands for all positive isolates were within the range of the gene's predicted size (200 bp)—figure 4

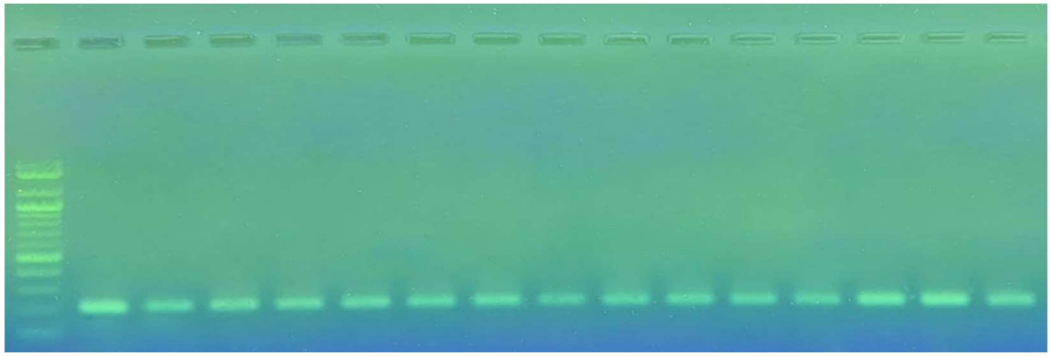

Figure (4) Traditional PCR amplification fragments (200 bp) for OmpX gene identification. Lanes 1–15 contain Enterobacter cloacae, a 100-bp DNA ladder, and negative control (NC). After 1.5 hours of electrophoresis on agarose gel (1.5%) at 70 V/cm, amplicons were examined using a UV transilluminator documentation system.

This study, which was conducted for the first time in Al-Anbar Governorate, revealed the detection of *Enterobacter cloacea* Complex genes . The results showed that OmpX gene were present in all

fifteen ECC isolatese. Overproduction of OmpX can lead to antibiotic resistance, and the presence of this protein may indicate pathogenic potential (17) . In Gram-negative bacteria, OmpA porins are extremely prevalent OMPs that play a variety of pathogenic activities, including adhesion, invasion, biofilm, serum resistance, evading host defenses, and antimicrobial resistance (18).

The results showed that fourteen isolates produced strong biofilms and only one(No.11) had weak biofilms (19,20)

Conclusion:

This thesis sheds light on the *Enterobacter cloacae* complex in particular. It showed its resistance to antibiotics and the possibility of establishing multidrug resistance, which makes it a severe nosocomial infection in intensive care units. This study also revealed outer membrane proteins. Where it detected *OmpX,* might imply that E. cloacae play a part in bacterial pathogenicity in addition to the role of other genes' adhesion, invasion, biofilm formation, and antimicrobial resistance.

Conflict of interest : No conflict of interest, **Funding**: Self

Ethical Clearance: This study is ethically approved by the Institutional ethical

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