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Molecular and susceptibility Study of Antibiotic Resistance Genes in *E. coli* Isolated from Selected Iraqi Patients

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ABSTRACT

A number of mobile genetic elements, such as plasmids and transposons have recently been shown, to promote the exchange of genetic material between genera and bacterial organisms. The purpose of this research was to investigate the prevalence and diffusion of resistance genes in the sample community (n=100). It was found that at least one group of antibiotics had been resisted in 62% of the study population, a multi-resistance phenotype was seen in 22% and no resistance to any form of compounds was observed in 16%. In all isolates exhibiting Ampicillin resistance, the presence of β-lactamase TEM was observed. 12 of the isolates were identified for the gene encoding OXA. The existence of a CTX-M chloroidal lactamase was tested in 12 isolate compounds with the upstream insertion sequence IS*Esp*1. There are 17 of the isolates that have an *int*1 gene. This study concluded that ampicillin and cephalosporin, first generation, have demonstrated the greatest resistance to the use of these antibiotics as a single agent for the treatment of infections. The prevalence of different forms of osteoarthritis in the population of the sample, on the other hand, indicates the need for regular monitoring of the distribution of such determinants of resistance. The CTX-M-15 example of plasmid mediated β-lactamases is alarming, given the simple discrimination between the same species or different species. The situation in these species is severe. The high incidence of resistant paediatric strain and multidrug-resistant strain in this same population is, after all, worried and needs close monitoring, because the care of selected patients is not recorded in the Baquba Teaching Hospital, Iraq, by many of the antimicrobials used.

Keywords: Resistance genes, Multidrug resistance, β-lactamases, Baquba Teaching Hospital

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INTRODUCTION

The emergence of antibiotic resistance at the community and hospital level is considered by many to be one of the most important threats to human health in the 21st century. Resistance to antibiotics is thus a serious public health problem worldwide, which translates into an inevitable increase in morbidity and mortality caused by infectious diseases, resulting in a reduction in the quality of life and an increase in health and care sectors[1]. In the mid-1940s, two years after the introduction of penicillin on the market, the first strain of penicillin-resistant *Escherichia coli* & *Staphylococcus aureus* appeared[2]. As a result of the diversity of environments that colonize and the characteristics of those environments, genes have been identified that confer selective advantages to the bacteria that possess them[3]. The inappropriate and indiscriminate use of antibiotics in treatments forces these microorganisms to adapt or die, and this phenomenon is called "selective pressure"[4]. The bacteria that manage to resist and survive in these conditions, carry antibiotic resistance genes, which makes the use of the respective antibiotic ineffective. Antibiotics, such as glycopeptides, quinolones, aminoglycosides and cephalosporins have also been used in animal feed, as prophylactics of infections and as growth promoters. In animals, resistant strains can also be selected, which easily act as a reservoir of resistance genes, contributing to the entry of multiresistant bacteria into the food chain and the environment[5]. In view of this scenario, the best option will be to use antibiotics when absolutely necessary, restricting their application in livestock. In a hospital environment, there are several factors that lead to a rapid appearance of resistance. *Escherichia coli* are a Gram-negative bacillus belonging to the Enterobacteriaceae family. It has simple nutritional requirements, is aerobic / anaerobic optional and has a biochemical profile characterized by fermenting lactose, reducing nitrates to nitrites, producing indole, having lysine and ornithine decarboxylase and being oxidase-negative[6] , being the main agent of low urinary infections (> 80%) due to the proximity between the gastro-intestinal tract and the urinary tract. However, it can ascend and cause pyelonephritis, enter the bloodstream (bacteraemia) or because infection located elsewhere. It is also associated with hospital-acquired pneumonia, septicemia, abscesses and gastroenteritis, however, until recently, this agent was not highly valued since it is an intestinal diner[7]. Antibiotics belonging to the same class have a similar mechanism of action and spectrum of activity; they generally share the same type of resistance and are also similar in terms of toxicity. An ideal antibiotic exhibits selective toxicity. Selective toxicity is often more relative than absolute, this implies that the drug in a concentration tolerated by the host organism has an effect on the microorganism causing the infection. Antimicrobial activity can be measured in vitro, by determining the Minimum Inhibitory Concentration (MIC) [8]. For a reliable test result, it is necessary to work with a standardized methodology proposed by Vassallo et al (2018)[9]. The sensitivity pattern of some bacterial species such as *pneumococcus, gonococcus, meningococcal* and *Haemophilus influenzae* is relatively predictable. Anti-infectious drugs in general and antimicrobials in particular have demonstrated an unquestionable efficacy in the treatment of infections, and their therapeutic utility is indisputable[10]. Bacteria can use individually or in combination several mechanisms to prevent the action of a certain antibiotic, such as: alteration of the antibiotic's action site; decreased intracellular concentration of the antibiotic, preventing its entry, by waterproofing the cell membrane, or exporting

the antibiotic, through efflux pumps; antibiotic inactivation, which in most cases occurs by enzymatic action, which is the main mechanism of resistance to β-lactam antibiotics. The resistance of a given microorganism to a given drug can be classified initially as intrinsic or acquired. Molecular typing, which is very important in biodiversity studies, also finds applications in Clinical Microbiology, namely in Epidemiology, given its ability to clarify the origin and spread of a strain that causes an infectious[11]. The results of molecular typing allow, among others, to observe the spread of multi-resistant bacteria to intra and inter-hospital antibiotics and to detect outbreaks caused by these bacteria. In fact, these often result from exposure to a common source of the etiologic agent and from its rapid spread among the potentially host population[12]. Genetically related organisms share genetic, biochemical and, often, virulence factors. The major goals of the current research are the identification in clinical isolates of genetic determinants of antibiotic resistance. *E.Coli* obtained from Baquba Teaching Hospital, Iraq to classify and evaluate the prevalency of extended spectrum beta lactamase (ESBL) strains and multi-resistant strains in the studied isolates of genetic variability, and the genetic variability of the 100 isolates.

MATERIAL AND METHODS

Bacterial isolates: 100 *E. coli* isolates were collected between December 2007 and February 2008. The isolates came from various departments of Baquba Teaching Hospital, Diyala Governorate, Iraq. The isolates were identified using the automated bacterial identification system (VITEK®2 COMPACT) instrument, in the Microbiology section of the Baquba Teaching. The isolates were obtained from several biological products: urine (*n = 81*), blood culture (*n = 8*) and other products (*n = 11*), including exudates and sputum. The strains were preserved in Tryptic Soy Agar (TSA) plates, stored at 4ºC and by freezing at -70ºC, in nutrient broth with 10% glycerol added. The collected isolates, after being obtained in pure culture and the respective Gram, were identified in the VITEK®2 Compact system. The susceptibility of clinical isolates to *E. coli* to the different classes of antibiotics was also performed in the VITEK®2 compact AES system. The antimicrobial agents tested are given in Figure 3. After 18-20h of incubation at 37ºC, a suspension was prepared, starting from a pure culture, in saline medium, with a density equivalent to 0.5 on the MacFarland scale. The identification galleries (GN) and the respective antimicrobial sensitivity test (N060) were inoculated, which were incubated for 18 hours.

Determination of the minimum inhibitory concentration: The ETEST method[13] (AB Biodisk) was applied to determine the minimum inhibitory concentration (MIC) of the following antibiotics: Imipenem, Ertapenem and Tigecycline.

Detection of broad-spectrum β-lactamases: The search for ESBL was initially carried out on the 100 isolates by the VITEK2 compact system and later confirmed positive by the Etest® method. The synergism test is easy to interpret. Thus, two commercially available strips of Etest® were used for the detection of ESBL. One of the strips contains increasing concentrations of ceftazidime (0.5µg / ml to 32µg/ml) at one end and increasing concentrations of ceftazidime (0.064µg/ml to 4µg/ml) associated with a fixed concentration (4µg / ml) of clavulanic acid at the other end. The other strip used contains cefotaxime in increasing concentrations (0.25µg / ml to 16µg/ ml) at one end and, at the other end, increasing concentrations of cefotaxime (0.16µg /ml to 1µg/ml) associated with a fixed concentration of clavulanic acid (4 µg / ml). A sample is considered to produce ESBL when there is a reduction in the minimum inhibitory concentration of ceftazidime>3 logarithmic dilutions in the presence of clavulanic acid compared to the MIC of ceftazidime without the same, that is, when the ratio between the MIC of the antimicrobial tested and the MIC of the antimicrobial associated with clavulanic acid In cases where both MICs showed values below the antimicrobial gradients present in the tape, the reduction was considered zero and the test was negative. On the other hand, the test was considered indeterminate when both MICs were above the tape scale, thus making it impossible to calculate the MIC reduction. From a bacterial culture with 18 to 20 hours of incubation at 37ºC, a suspension was made with sterile distilled water with a turbidity of 0.5 on the McFarland scale. It was inoculated in Petri dishes with Mueller Hinton agar (Oxoid), sowing with a swab.

Amplification of DNA fragments by PCR: From a culture with an incubation period at 35ºC of 18-24H, in soybean trypticase agar, two colonies were removed, which were suspended in 200µL of sterile distilled water. From this suspension, 1 µl was removed for the PCR reaction. All PCR reactions (Table 1) were prepared to a final volume of 50 µL and were run on a Thermo Scientific-ProFlex PCR System.

Search for Integrations: For the search for class 1 integrations, the primers described in Table 1 were used. In Table 1, the conditions of the amplification reactions are indicated.

Table 1: Indication of the final concentration of the components of the PCR reaction

Reagentes	Final concentration	Volume (µL)
25 mM $MgCl_2$	3.0 mM	6
5 x Buffer	1 x	2.5
2 mM dNTP mix	0.2 mM of each	5
DMSO	5%	2.5
Initiator 1	0.3 pmol / ml	1.5
Initiator 2	0.3 pmol / ml	1.5
Total DNA	Variable	variable
Taq (1 U/µL)	1 U	1
dH_2O		to make a volume of 50.0 µL

Screening for ß-lactamases of the OXA, TEM, CTX-M type: The search of genes for encoding β-lactamases TEM, OXA and CTX-M was carried out by PCR on all samples. Table 2 & 3 below explains the oligonucleotide primers used to amplify every gene, as well as the conditions used in the PCR reactions.

Table 2: Sequence of the primers to search the different families of ß-lactamases, size of the respective amplicon and respective reference

Initiator Designation	Sequence	Target gene	Fragment size	Reference
TEM-F	5'-AAA TGC TGA AGA TCA-3'	*bla*TEM	425bp	14
TEM-R	5'-TTT GGT ATG GCT TCA TTC-3'	*bla*TEM	1076bp	15
TEM-F	5'-TTACCAATGCTTAATCA-3'			
TEM-R	5'-CTCGTCGTTTGGTATGGC-3'	*bla*CTX-M	400bp	16
CTX-F	5'-GTGCAGTACCAGTAAAGTTATGG-3'			
CTX-R	5'-CGCAATATCATTGGTGGTGCC-3'	*bla*OXA	814bp	17
OXA-F	5'-ACACAATACATATCAACTTCGC-3'			
OXA-R	5'-AGTGTGTTTAGAATGGTGATC-3'			

Table 3: Conditions used in the amplification reactions of the different families of ß-lactamases.

Phases	Program*		
	TEM	OXA	CTX-M
Initial denaturation Denaturation Primer hybridization Synthesis	90°C	90°C	90°C
	90°C	90°C	90°C
	34°C	37°C	39°C
	68°C	68°C	68°C
Final synthesis cycles After reaction	30 Times	30 Times	30 Times
	68°C	68°C	68°C
	14°C	14°C	14°C

Time: 30 to 40 Minutes

Table 4: Sequence of primers to search for the class 1 integrate coding gene, of the variable zones, size of the respective amplicons and reference

Primer designation	Sequence	Gene to be amplified	Fragment size	Reference
HS 463a	5'-CTGGATTTCGATCACGGCACG-3'	*IntI* 1	500 bp	[18]
HS 464	5'-ACATGCGTGTAAATCATCGTCG-3'			
RB 317	5'-GAACCTTGACCGAACGCAG-3'	*Zv* 1	Variable	
RB 320	5'-AGCTTAGTAAAGCCCTCGCTAG-3'			

Table 5: Conditions used in the amplification reactions of class 1 integrals and respective variable zones.

	Programme	
PCR phases	*IntI*1 Integrase*	Variable zone 1
Initial denaturation	90°C	90°C
Denaturation	90°C	90°C
Primer hybridization	66°C	66°C
Synthesis	68°C	68°C
Cycles	30 Times	30 Times
Final summary	68°C	68°C
After reaction	14°C (Long Time)	14°C (Long Time)

Time: 30 to 40 Minutes

Agarose gel electrophoresis and DNA visualization: The PCR products were separated by agarose gel electrophoresis, with a concentration of 1%, in buffer (TAE) 1X, to which was added bromide ethidium at a final concentration of 10mg / ml. The sample volume applied to the gel was 1/10 of the reaction volume, to which 1x concentrated loading buffer was added.

Electrophoresis was carried out at 80V, for 1 hour and 30 minutes, in TAE 1X buffer: In all electrophoresis performed, DNA molecular weight markers were introduced. The size of the DNA fragments was determined by comparison with the migration of those of fragments of known molecular weight[19]. In the present work, the following markers were used: *Sau3*AI marker and *lacZ* gene.

Sample preparation, determination of the nucleotide sequence and analysis of the sequenced fragments: The purification of the amplification products was performed. The nucleotide sequences of the purified PCR products were determined, in both orientations; the resulting nucleotide sequences were analyzed with the BLAST program[20] and compared with other sequences deposited in the databases.

Study of genetic variability: For the study of genetic variability, the rep-PCR technique was used, with the primers and PCR reaction conditions described in tables 6 and 7.

Table 6: Sequence of primers for amplification by ERIC-PCR

Reaction Type	Primer designation	Respective sequence	Reference
ERIC - PCR	ERIC 1	5'-ATGGCTTAACCTGATTGGGCAC-3'	21
	ERIC 2	5'-AAGTTGACAAGTGCGGGGGTGA-3'	22

Table 7: Conditions of the amplification reactions for ERIC-PCR

Phases	Program
Denaturation	95 º C 7 '
initial Denaturation	94 º C 1 '
Hybridization of primers	52 º C 1 '
Summary of chains	65 º C 8 '
Cycles	35 Times
Final summary	65 º C 16 '
After the end of the reaction	15 º C ∞

Electrophoresis Conditions: The PCR products were separated by electrophoresis on agarose gel (1.5%, Sigma), in 1X buffer (TAE), to which ethidium bromide was added at a final concentration of 10mg / ml. The sample volume applied to the gel was 1/10 of the reaction volume, to which 1x concentrated loading buffer was added. Electrophoresis was carried out for 3.5 hours at 100V.

RESULTS AND DISCUSSION

Bacterial isolates: Collections from the same patient, but with a minimum of seven days apart, were considered to be a different isolate. Of these isolates, 17 came from the external consultation, 59 from the emergency room and 24 from the various inpatient services at the Baquba Teaching Hospital, Iraq. Figure 1 shows the distribution of the 100 *E. coli* isolates collected, between September 2018 and January 2019, from the different patients, according to age groups. Figure 2 shows the biological products from which the strains selected for the present study were isolated.

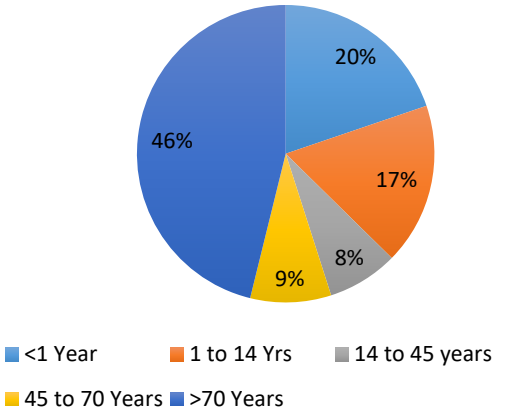

Figure 1: Graphical representation of the studied clinical isolates, distributed by different age groups

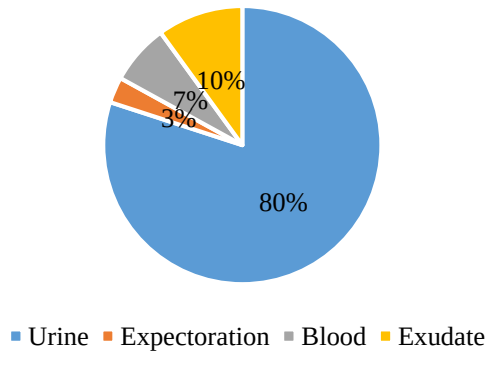

Figure 2: Origin of the *E. coli* clinical isolates selected for the present study.

EPIDEMIOLOGICAL DATA

Susceptibility to antimicrobials of *E. coli* isolates: Figure 3 shows the resistance profile to different antibiotics, found in the isolates selected for this study.

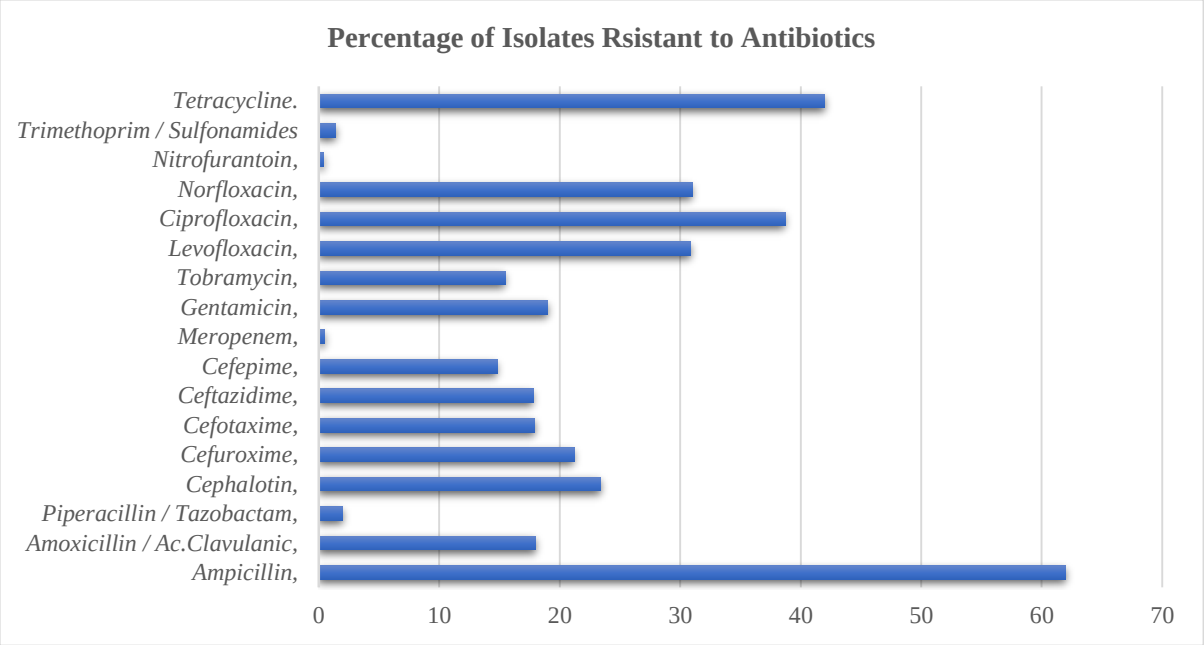

Figure 3: Antibiotic resistance profile of the studied clinical isolates. The X axis represents resistance values translated into present and the Y axis represents the tested antibiotics.

Several authors suggest that high levels of resistance to antibiotics are related to a longer therapeutic availability of an antibiotic, such as ampicillin and tetracycline[23]. In the present study, it was found that ampicillin was the ?-lactam that presented a higher level of resistance, which was verified in 64% of the isolates (Figure 4).

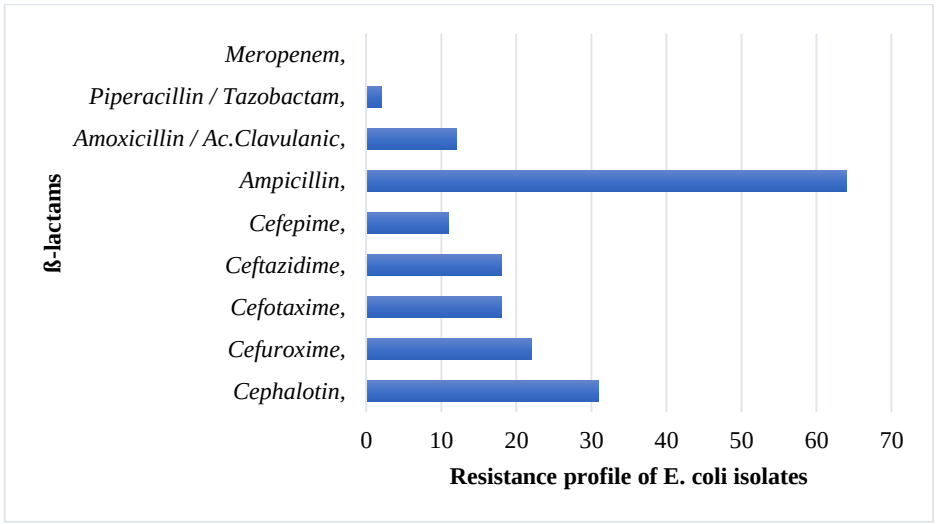

Figure 4 - Resistance profile of *E. coli* isolates to ß-lactam agents, including penicillins and association with ß-lactamase inhibitors, cephalosporins and carbapenems.

In none of the isolates, reduced susceptibility to meropenemus was detected, which is consistent with recent studies[24] (Figure 4). Meropenem, representative of carbapenems, thus constitutes the only effective antibiotic against all isolates, consequently the therapeutic option in systemic infections. However, its therapeutic efficacy is threatened by the production of metallo-beta-lactamases, namely by the Pseudomonaceae family[25]. With regard to cephalosporins, it was possible to observe a resistance gradient when analyzing the classes of these antibiotics. The highest percentage (31%) of resistance was to cephalothin (first generation cephalosporin) and the lowest percentage (11%) to cefepime (fourth generation cephalosporin). In third generation cephalosporins, such as cefotaxime and ceftazidime, it was found that the isolates were resistant to both drugs simultaneously (n = 18). Xia et al. (2013)[26] considered multiresistant bacteria (MRB) to be those that express resistance to two or more classes of non-beta-lactam antibiotics (aminoglycosides, fluoroquinolones, nitrofurantoin and / or trimethoprim) Considering these criteria, 30 isolates of the present study were considered to be multidrug-resistant, of which 17

came from the community and the rest from hospitalized patients. With regard to quinolones, 39% of the isolates were resistant to Ciprofloxacin. The resistance verified in relation to this class is worrying, since there was no significant difference between resistance to third generation and fourth generation quinolones. In 50% of these isolates, resistance to cephalosporins was concomitantly observed. Recent epidemiological surveillance studies, namely, carried out by Kareem et al. (2015)[27], illustrate the alarming association of resistance to various classes of antimicrobial agents among strains producing ß-lactamases from the community. In aminoglycosides the lowest percentage of resistance was observed in relation to Amikacin (1%). Tobramycin and gentamicin showed 14 and 19% resistance in the studied isolates. The same has been observed by other authors in previous studies.[28]

Detection of broad-spectrum -lactamases: In the isolates reported as positive for the presence of ESBL (21%), this was later confirmed by strips of Etest-ESBL CT/CTL, TZ/TZL and PM/PML, the results being concordant. Resistance to 3rd generation cephalosporins resulting from the acquisition and expression of ESBLs is well documented[29]. The clinical implications of this are numerous and serious, and therefore the rapid detection of ESBL-producing strains is essential for the application of appropriate therapy and for monitoring the development of resistance. As the PCR-based methods are not yet a hospital routine, Etest- emerges as the method of choice for the rapid detection of ESBL-producing strains. It should be noted that of the 21% of isolates where the research was positive for ESBL, five come from the Paediatrics service, which once again highlights the growing concern regarding the dissemination of these determinants of resistance in increasingly younger age groups.

Research of genes coding for ß-lactamases by PCR

TEM, OXA, CTX-M TEM research: Figure 5 shows the result of an electrophoresis of the amplicons resulting from a polymerase chain reaction with specific oligonucleotides (Table 2) for ß-lactamases of the TEM type. In 64 isolates, an amplicon with a size of approximately 425 bp was obtained.

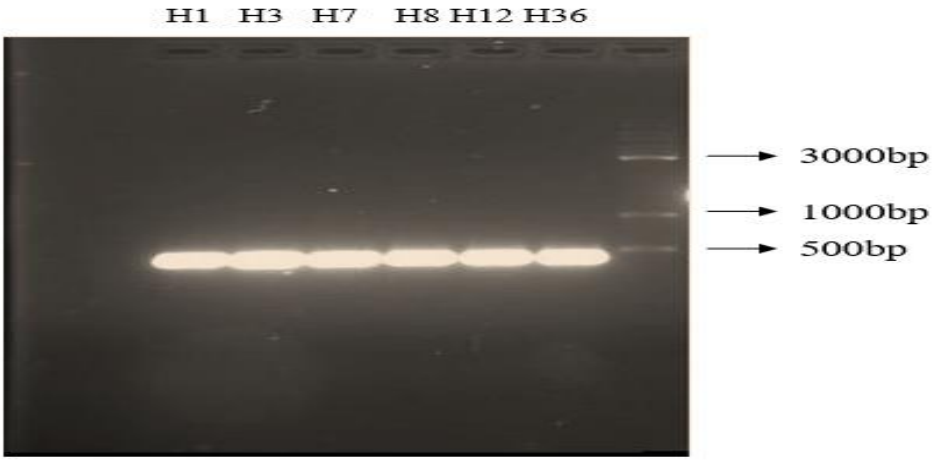

Figure 5: Image of an agarose gel of the products resulting from the amplification of the *bla*TEM gene.

In all isolates in which the *bla*TEM gene search was positive, resistance to ampicillin was observed, suggesting a strong correlation between the results obtained. The analysis of the obtained nucleotide sequences revealed high homology with the *bla*TEM-1 gene, which, as expected, was the predominant gene among the population studied. In fact, previous studies have shown that β-lactamases TEM-1 / -2 are widely disseminated among Gram negative bacilli and constitute the main mechanism of resistance to reduced-spectrum penicillins.[30]

OXA: Regarding the *bla*OXA gene search, the obtained amplicon was the expected size (814 bp) (Figure 6) and was present in 12 isolates. After determining the nucleotide sequence, it was found that all showed high homology with *bla*OXA-30. Based on studies previously carried out, it is an enzyme with special hydrolytic activity against cloxacillin and amoxicillin.[31] Among the *bla*OXA genes found, only one is located in a class 1 integration. This fact, described in the literature, warned of the possibility of more easily the spread of this gene among other isolates.[32]

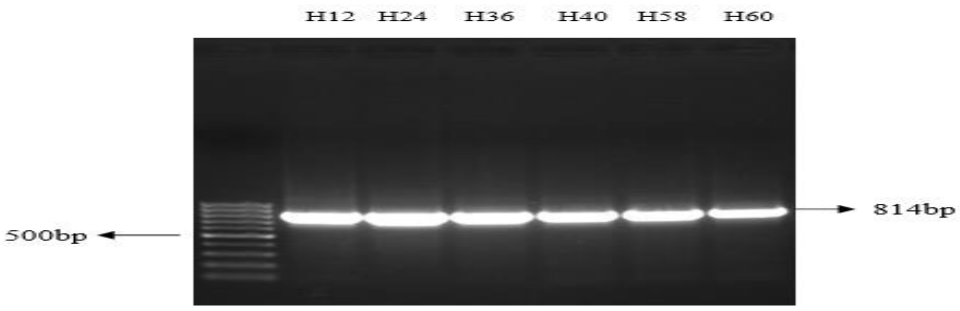

Figure 6: Photograph of an agarose gel of the products resulting from the amplification of the *bla*OXA gene.

CTX-M: In the search for the *bla*CTX-M gene, amplification products were obtained in twelve isolates. The amplicon was 528bp in size, as expected. This gene, contained in a plasmid, encodes enzymes that are characterized by preferentially hydrolyzing. The recent spread of this gene among several species poses a threat to the efficacy, in the clinic, of 3rd generation cephalosporins. Thus, it was found that all of these isolates (n = 12) had resistance to cefotaxime, that is, they had minimum inhibitory concentrations (MICs) equal to or greater than 64 mg / l. A study conducted by Fatima Abbas (2019)[33] in Iraq, shows that strains producing CTX-M are more resistant to different classes of antibiotics than bacteria that produce other types of ESBL. In the present study, it was found that among the strains producing CTX-M, 75% (n=9) were resistant to quinolones and 42% (n= 5) resistant to tetracycline. The genes encoding β-lactamases are usually associated with insertion sequences (IS), mainly in Enterobacteriaceae and *Pseudomonas aeruginosa*. The PCR reaction with specific primers allowed the detection of the insertion sequence upstream of all *bla*CTX-M genes.[34] Several authors[35] have shown that this element has the ability to mobilize the *bla*CTX-M gene, thus constituting a key factor in its dissemination.

Search for Integrations: From the search for the class 1 (*intl*1) integrase gene in the isolates of this study (Table 8), 17 were positive, which indicates that only these have one or more class 1 integrations. The bacterial suspensions of the positive *E. coli* isolates were later submitted to a new PCR to amplify their variable regions. The presence of 6 different gene cassettes with sizes ranging from 800 to 2000 bp was detected in 15 of the 17 isolates. The predominant genes in the arrays found were variants of the aadA gene, which confer resistance to streptomycin and spectomycin. These tapes represent 50% of the total tapes found and include aadA1, aadA2 and *aad*A5. Although streptomycin and spectomycin are antibiotics rarely used in therapy, *aad*A gene cassettes are prevalent in varying zones. According to Meteab Alshammari (2019)[36], the prevalence of these genes precludes the possibility of integrase being able to excise them. However, in our present study it was not possible to detect integrations in all samples that exhibited resistance to cotrimoxazole. Thus, the hypothesis of the presence of class 1 integrations that escape the classic amplification procedures of the variable zone must be considered. Given the resistance observed in the remaining isolates, and since it does not appear to be encoded in genes that were amplified in the isolates under study, it appears that there are other resistance mechanisms encoded by genes not associated with mobile elements.

Table 8: Positive isolates for the presence of class 1 integrons, amplified cassette genes and their size.

Isolated	Genes Cassettes	Array size (bp)
H9	***dfrA1-aadA1***	**1900**
H15	***dfrA1-aadA1***	**1900**
H40	***bla$_{OXA30}$ –aadA1***	**2300**
H42	***dfrA25;***	**800**
	dfrA12-aadA2	**2200**
H46	***spec-aadA1***	**1200**
H49	***dfrA17-aadA5***	**2000**
H57	***dfrA17-aadA5***	**1900**
H63	***dfrA17-aadA5***	**1800**
H65	***dfrA1-aadA1***	**1800**
H82	***dfrA1-aadA1***	**2200**

Study of genetic variability: Figure 6 shows the amplification profile of the 100 *E. coli* isolates using ERIC primers. This amplification was only positive for 88 of the isolates under study. The number of bands varied from 8 to 12 per isolate, and 6 clones as described below were identified.

H2 / H4: Of the clones found, it was subsequently found that H2 / H4 were isolated from the same patient two days apart and were therefore excluded from the study, since it was based on a criterion that established a minimum of 7 days apart for consider different isolates.

H36 / H7: When analyzing the provenance of H36 / H7 clones, it was found that H7 was isolated from a 69-year-old male patient and H36 was isolated from a 67-year-old female patient, both from the community. Analysing its resistance profile, it was found that it was quite different, with the isolate H36 presenting a greater number of resistances. When comparing the content of genetic determinants of mobile resistance that both isolates had, it was clearly found that the more resistant isolate (H36) had more mobile elements. Isolate 36 was positive for the presence of *bla*CTX-M-15, which according to the literature would justify its resistance to 3rd generation cephalosporins. The H36 isolate was also positive for the presence of *bla*OXA30, not making this part of the variable zone of a class 1 integration as is often described. Thus, as already reported by other authors, this gene would be associated with an element that allows its mobilization. In none of the isolates (H7 / H36) was there the presence of the *Int*1 gene which codes for the integrase and therefore would be indicative of the presence of class 1 integration.

H82 / H60: Similarly, H82 / H60 clones were also isolated from elderly patients of different sexes and both from the community. Regarding its resistance profile, this also proved to be quite different, with the H60 isolate presenting a greater number of resistances. Also in the

case of the H60 isolate, the presence of the *bla*CTX-M gene associated with the IS*Ecp*1 insertion sequence was verified, and as already mentioned above, in this case, this association of genetic structures was found in an isolate from the community. Like the one found in the H36 isolate, also in the case of the H60 isolate, the presence of the *bla*OXA gene was found, and also in this case this gene was not part of the variable zone of a class 1 integration, and it is suggested that it may be linked to a mobilization gene. As described for the previous pair, the Int1 gene was not amplified in the H82 / H60 isolates.

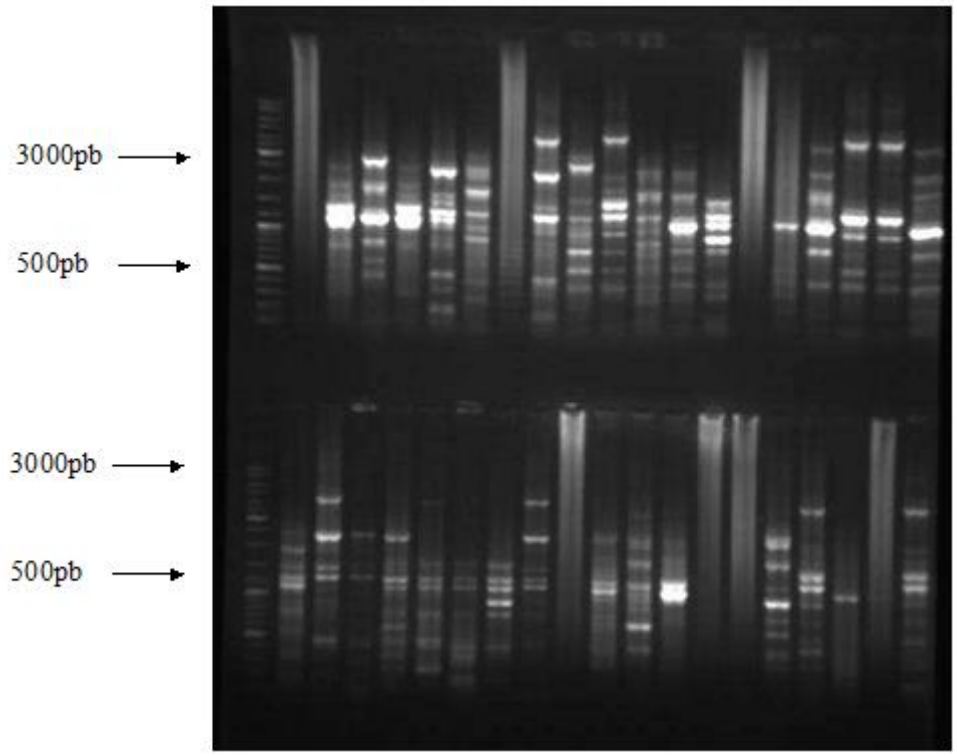

Figure 6- Electrophoretic profiles of the fragments obtained after PCR amplification with the ERIC primers.

In general, and through the analysis of the dendrogram obtained based on the electrophoretic profiles it was possible to conclude that there is a great genetic variability among the isolates under study. Only 3 pairs of clones (H36 / H7; H82 / H60; H2 / H4) showed 100% homology. In about 16 isolates there was homology greater than 80%, with the remaining isolates showing lower homology.

CONCLUSION

1. Ampicillin and first generation cephalosporins were observed to have the highest degree of resistance as expected. These antibiotics are not meant to be used as a single agent in *E coli* care, as a result. Infections of bacteria. 21% of isolates have also been found to be susceptible to all antibiotics studied.
2. The set of gene cassettes found in the study population demonstrates, once again, the ease with which currently resistance genes can be disseminated. The *dfr*A17-aad A5 array example is illustrative of this. This "array" of genes identified in the study population, has previously been described in different bacterial genera from different origins and in different parts of the globe.
3. The presence of different types of broad-spectrum β-lactamases in the study population demonstrates the importance of using methods for detecting ESBL-producing isolates in the hospital routine in order to minimize therapeutic failures. The present study also confirms the spread of *bla*CTX-M-15 in the hospital environment (Baquba Teaching Hospital, Iraq) and in the community, similarly to what occurs at the national level. Since these plasmid-mediated beta-lactamases, this represents an epidemiological problem, since these elements can be mobilized in between strains and species.
4. Special attention should be paid to the high prevalence of resistant *E. coli* strains in paediatrics and the rise of multi-resistant strains in this specific population. As the treatment of urinary tract infections in such patients may be jeopardised, many anti-microbial agents used for pathogens are not suggested.
5. There was a great genetic variability among the isolates studied, a fact that may be related to a large share of the isolates that came from the community. The results obtained suggest an inversion in the flow of multidrug-resistant strains, as has been reported by the scientific community, and there is currently an import of multidrug-resistant strains from the community into the hospital environment.

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