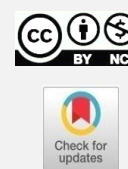




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RESEARCH ARTICLE

PREVALENCE OF *BLATEM*, *BLASHV*, AND *BLACTX-M* GENES AMONG ESBL-PRODUCING *ESCHERICHIA COLI* ISOLATED FROM THE BLOOD SAMPLES OF ICUS PATIENTS OF UNIVERSITY HOSPITALS IN SANA'A CITY, YEMEN

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Abstract

Aim and Objective: With the emergence of organisms such as *Enterobacteriaceae* that produce extended-spectrum β -lactamase (ESBL), which are resistant to multiple medications (multidrug-resistance), concerns about how best to treat infections have significantly increased. The current study examined the molecular features of ESBL in clinical isolates of *Escherichia coli* that resulted in bloodstream infections as well as the pattern of antibiotic resistance to gather useful data on the infection's epidemiology among Yemeni ICU patients.

Subjects and methods: A cross-sectional study was conducted on sepsis patients admitted in intensive care units at four hospitals in Sana'a, Yemen, between January, 2021 and April, 2022. Blood cultures were used on patients suspected of having sepsis. Standard laboratory procedures were then used to isolate and identify possible bacterial infections, and the disk diffusion method was used to test for microbial susceptibility. All strains were tested for Extended Spectrum Beta-Lactamase (ESBL) production using the Modified Double Disc Synergy Test (MDDST). Following analysis, polymerase chain reaction, β -lactamase genes (*blaTEM*, *blaSHV*, and *blaCTX-M*) were identified.

Results: The results of the conventional PCR experiment revealed that 33.3% of the *blaCTX-M* genes, 0.0% of *blaSHV*, and 100% of *blaTEM* were present in the strains of ESBL-producing *E. coli* that were collected. It was discovered that the *E. coli* isolates' patterns of antibiotic resistance to 23 different antibiotics differed greatly. The bulk of the *E. coli* isolates were found to be multi-drug resistant (MDR). Furthermore, MDR characteristics were observed in 85% of *E. coli* isolates.

Conclusion: Control and surveillance of antibiotic resistance depend on an understanding of the resistance genes and patterns of antimicrobial resistance of bacterial pathogens within a given geographic area. The current study's findings showed that MDR was very common. According to the current study's findings, TEM was significantly more common than other ESBL gene types.

Keywords: *blaCTX-M*, *blaSHV*, *blaTEM*, Blood stream infections (BSIs), ESBL, *Escherichia coli*, ICUs, Multi-drug resistant (MDR).

INTRODUCTION

Numerous nosocomial illnesses worldwide have been documented to have bacteria from the *Enterobacteriaceae* family as their causative cause. Given the limited therapy options resulting from the organisms'

ongoing rise in antibiotic resistance, infections caused by bacilli *Enterobacteriaceae* are challenging to control. Indeed, one of the most well-known resistance mechanisms in Gram-negative bacilli was first described by Ojdana *et al.*¹, and involves ESBLs. A class of enzymes known as ESBLs increases the

resistance to cephalosporins, penicillins, related β -lactams, ceftazidime, and acetaminophen-olactams, however clavulanic acid inhibits ESBLs¹. The three primary categories of ESBLs are TEM, SHV, and CTX-M. The rapidly growing CTX-M family, which is now more common than SHV and TEM, is found in a wide variety of clinically significant bacteria and over large geographic regions². Treatment plans become more complicated because organisms that produce ESBL frequently show resistance to antibiotics from other classes, such as quinolones, aminoglycosides, and sulfonamides³. Furthermore, members of the *Enterobacteriaceae* family, like *Escherichia coli*, frequently produce ESBLs; yet, it has recently been shown that some additional enzymes may be present in other *Enterobacteriaceae* family genera. It was initially discovered that patients in European intensive care units who had extended hospital stays exhibited a greater degree of resistance in these organisms. Nevertheless, isolates were found in South and North America, Africa, Asia, the Middle East, and Africa, and ESBL GNB quickly spread around the world⁴. Common ESBL genes that code for *E. Coli* isolates were identified as TEM (discovered and isolated in the early 1980s from Teminora, a Greek patient), and CTX-M (cefotaximase that preferentially hydrolyzes cefotaxime). There are occasional descriptions of these transposon- plasmid- and chromosome-mediated genes all over the world⁵.

Every year, the rates of bacterial resistance rise, raising concerns around the world. For this reason, it is crucial to understand susceptibility patterns because improper empirical antimicrobial therapy can lengthen hospital stays and increase mortality rates, both of which can be prevented with the right therapy⁶. Acquiring additional PBPs in sensitive to β -lactam or changing the normal PBPs are known as the commonest cause of resistance in cocci such as MRSA and *pneumococci* which are gram positive. However, a mixture of endogenous acquired β -lactamases with natural efflux and up-regulated impermeability is the main reason for resistance in the gram-negative bugs⁷. It should be mentioned that there are well-written materials demonstrating how standard disc diffusion tests are unable to identify the development of ESBLs. There might not be enough resources in labs to stop the spread of these resistance mechanisms since many clinical laboratories do not completely understand the importance and detection technique of ESBLs⁸.

There are many different types of ESBLs, such as SHV, TEM, OXA, CTX, AmpC, and so on; however, the majority of them are derived from the SHV, TEM, and CTX-M enzymes, which are most frequently present in *E. coli*. In light of this, the current work examined the presence of *bla*SHV, *bla*CTX-M, and *bla*TEM genes in isolates of *E. coli* from the bloodstream of intensive care unit patients at tertiary hospitals in Sana'a, Yemen, in order to ascertain the prevalence of the ESBL phenotype.

SUBJECTS AND METHODS

Isolates of bacteria: Twenty consecutive non-duplicate *E. coli* isolates were recovered from blood culture specimens of ICU patients suffering from sepsis. A cross-sectional study was conducted on sepsis patients admitted in intensive care units at four hospitals in Sana'a, Yemen, between January, 2021 and April, 2022. Blood cultures were performed on patients suspected of having sepsis, and possible bacterial infections were subsequently isolated and identified using conventional laboratory procedures⁹. To identify the isolates, standard microbiological methods were used. Additionally, they were re-identified using the VITEK 2 compact system (BioMerieux, France).

Ethic approval: All of the techniques employed in this study were authorized by the research and ethics committee of the Faculty of Medicine and Health Sciences at Sana'a University, Sana'a, Yemen (Approval No. UGR/SU-223).

Antimicrobial susceptibility testing: The isolates were screened using the disc diffusion method (Kirby-Bauer disc diffusion method) on Mueller-Hinton agar (MHA) plates in accordance with the criteria of the Clinical and Laboratory Standards Institute (CLSI) to determine their antibiotic susceptibility¹⁰. Amoxicillin+Clavulanic acid (20+10 μ g), Amikacin (10 μ g), Azithromycin (15 μ g), Cefotaxime (30 μ g), Ceftazidime (30 μ g), Cefazoline (30 μ g), Cephadrin (30 μ g), Cefoxitin (30 μ g), Cefuroxime (30 μ g), Ceftriaxone (30 μ g), Cefoperazone (30 μ g), Cefepime (30 μ g), Co-Trimoxazole (25 μ g), Ciprofloxacin (10 μ g), Imipenem (10 μ g), Gentamicin (10 μ g), Meropenem (10 μ g), Norfloxacin (10 μ g), Moxifloxacin (10 μ g), Piperacillin (100 μ g), and Tobramycin (10 μ g).

Testing for production of ESBL (MDDST): A disc containing four cephalosporins (Ceftriaxone, 3GC-Cefotaxime, 4GC Cefepime, and Cefpodoxime) and amoxicillin-clavulanate (20/10 μ g) was used in the Modified Double Disc Synergy Test (MDDST) to assess each strain's ability to produce Extended Spectrum Beta-Lactamase (ESBL). On a Mueller-Hinton agar plate, a lawn culture of the organisms was established in accordance with CLSI guidelines¹⁰. Placing a disc in the center of the plate held 20/10 μ g of amoxicillin-clavulanate. The amoxicillin-clavulanate disc's center was positioned 15 mm and 20 mm from the center of the 3GC and 4GC discs, respectively¹¹. Any expansion or deformation in the zone toward the disc of amoxicillin and clavulanate was considered indicative of ESBL development. The combined disc test was used to validate ESBL production in accordance with CLSI recommendations.

Detection of ESBL genotypes by multiplex PCR amplification: Multiplex PCR was used to check for the presence of *bla*SHV, *bla*CTX-M, and *bla*TEM genes in the isolates that tested positive for ESBL production in the first screening test¹³. This approach was somewhat modified from that used by Monstein et al.¹². Using a PrestoTM Mini gDNA bacterial kit, freshly cultivated isolates of bacteria were utilized to prepare template deoxyribonucleic acid (DNA). 0.2

units/μl Ampliqon Taq DNA polymerase, 0.4 mM of each dNTP, 0.4 μM of each primer, and 2 μl DNA template (density of 10 ng/μl) were used in all PCR reactions. The Master Mix included 20 mM Tris-HCl pH 8.5, 0.2% Tween® 20, 3 mM MgCl₂, and (NH₄)₂SO₄. The following settings were made for the polymerase chain reaction amplification: a 10-minute

main denaturation step at 95°C; thirty denaturation cycles at 94°C for 30 seconds; annealing at 60°C for 30 seconds; an extension step at 72°C for two minutes; and a final extension step at 72°C for ten minutes. Size separation PCR amplicons were used in conjunction with agarose gel electrophoresis to identify the corresponding genes.

Table 1: The frequency of isolated bacteria from ICUs patient's blood cultures in the selected hospitals in Sana'a city.

Micro-organisms	No. (%)
Gram positive bacteria	42 (43.7)
Coagulase negative	25 (26)
<i>Staphylococci</i>	
<i>S. aureus</i>	9 (9.4)
<i>S. pneumoniae</i>	5 (5.2)
<i>Enterococci</i>	2 (2.1)
<i>S. pyogenes</i>	1 (1.0)
Gram negative bacteria	50 (52.1)
<i>E. coli</i>	20 (20.8)
<i>Klebsiella species</i>	11 (11.5)
<i>B. cepacia</i>	6 (6.3)
<i>H. influenzae</i>	5 (5.2)
<i>A. baumannii</i>	4 (4.2)
<i>P. aeruginosa</i>	3 (3.1)
<i>C. indologenes</i>	1 (1.0)

RESULTS

Twenty successive non-duplicate *E. coli* isolates were recovered in total, and tests were performed on their antimicrobial resistance profile against 23 distinct antimicrobial drugs. The current results revealed that *E. coli* isolates vary widely to different antimicrobials. The resistance rates of isolates of *E. coli* against the selected 23 antimicrobial agents obtained from blood of ICU patients. It was found that a majority of the *E. coli* isolates were resistant to several drugs (multi-drug resistant: MDR) where a total of 17/20 (85%) of *E. coli*

isolates indicated MDR phenotypes. Furthermore, the results of the antimicrobial susceptibility test against *E. coli* revealed that *E. coli* showed 45% resistance to Amoxicillin+Clavulanic acid (Table 2), whereas susceptibility to ciprofloxacin decreased to 35%. The highest sensitivity rate of *E. coli* was for aminoglycosides classes where it was 95% for amikacin and 90% for gentamicin. Whereas, the highest resistant rate was for the 1st, 2nd, 3rd and 4th generations of Cephalosporins β-lactam class (80%, 90%, 95%, 90%, 95%, 85% and 100%, respectively) (Table 2).

Table 2: The antibiotics susceptibility for the total 20 *E. coli* isolated from sepsis patients of ICU.

Antibiotics name	Classes	Sensitive No. (%)	Resistant No. (%)
Ampicillin	Penicillin/amino-penicillin	5(25)	15 (75)
Piperacillin- Tazobactam	Penicillin and β- lactamase	12(60)	8 (40)
Amoxicillin-Clavulanate	inhibitor	11(55)	9 (45)
Cefazoline		0(0)	20 (100)
Cefadroxil	1 st generation	1(5)	19 (95)
Cephadrin		4(20)	15 (80)
Cefoxitin	2 nd generation	2(10)	18 (90)
Cefuroxime		1(5)	19 (95)
Ceftazidime ESBL		2(10)	18 (90)
Cefotaxime ESBL		1(5)	19 (95)
Ceftriaxone	3 rd generation	3(15)	17 (85)
Cefoperazone		2(10)	18 (90)
Cefepime	4 th generation	2(10)	18 (90)
Imipenem	Carbapenems	14(70)	6 (30)
Meropenem		16(80)	4 (20)
Aztreonam	Monobactams	8(40)	12 (60)
Amikacin	Aminoglycosides	19(95)	1 (5)
Gentamicin		18(90)	2 (10)
Co-Trimoxazole	Folate pathway inhibitors	5(25)	15 (75)
Ciprofloxacin		7(35)	13 (65)
Levofloxacin	Fluoroquinolones	10(50)	10 (50)
Norfloxacin		6(30)	14 (70)
Moxifloxacin		8(40)	12 (60)

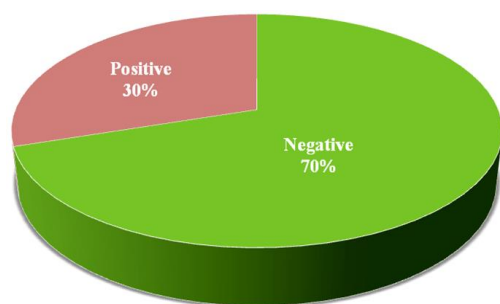


Figure 1: The ESBL producing *E. coli* (N=20) isolated from the blood sample of ICUs patients.

Co-trimoxazole with resistance rates 70%, and 70% respectively. Out of the 20 *E. coli* isolates, a total of 6 isolates (30%) showed positive results in initial screening test of ESBL production by MDDST and phenotypic confirmatory test of ESBL production. In PCR detection of ESBL genotypes, it was found that all of the ESBL screening positive *E. coli* isolates had one or more ESBL genes that were tested in the present study. Overall, 30% (6/20) of *E. coli* isolates were positive for one or more ESBL genes. The multiplex PCR assay results indicated that 100% *bla*TEM genes,

33.3% *bla*CTXM and *bla*SHV genes were not detected in the *E. coli* isolates.

DISCUSSION

Antimicrobial resistance in pathogenic bacteria is a global concern that is associated with elevated rates of morbidity and mortality. Furthermore, infections have been reported to be difficult or impossible to treat with traditional antimicrobials due to multidrug resistance patterns. Antibiotics are widely, generously, and usually needlessly utilized because many healthcare facilities fail to identify the underlying bacteria and their patterns of antimicrobial sensitivity in a timely manner in patients with bacteremia and other serious diseases¹³. High prevalence of MDR *E. coli* isolates was found in the blood clinical samples used in the current study. MDR traits were seen in 85% of the *E. coli* isolates overall. Thirty percent of the *E. coli* MDR isolates were ESBL producers. Unlike the findings of Bora et al.¹⁴, which stated that 75% of *E. coli* isolates produced ESBL. The findings of the test of antimicrobial susceptibility against *E. coli* in the current investigation showed that the susceptibilities of the isolated bacteria to the tested antimicrobials varied.

Table 3: The prevalence rate of ESBL genes of ESBL-producing *E. coli* isolated from the blood sample of ICUs patients.

ESBL genes	<i>bla</i> TEM No. (%)	<i>bla</i> CTX-M No. (%)	<i>bla</i> SHV No. (%)
Negative	0 (0)	4 (66.7)	6 (100)
Positive	6 (100)	2 (33.3)	0 (0)

Antimicrobial susceptibilities patterns were identified in all isolates. Similar findings were reported by Tabar et al.³, and Liao et al.¹⁵. Amino glycosides and carbapenems are frequently the last effective treatments for infections caused by MDR *Enterobacteriaceae*¹⁶. Imipenem and Meropenem, which have been reported to be the most effective

antibiotic, including the isolates that create ESBLs, showed 100% sensitivity, according to previous investigations. Given that carbapenems can be used to treat a variety of infections, this is a significant finding of the current study. This outcome may be explained by the fact that these antibiotics are less common in this area due to their higher cost.

Table 4: List of primers used for Multiplex PCR amplification.

Target gene	Primer	Sequence (5'-3')	Amplicon size bp	References
<i>bla</i> TEM	Forward	ATGAGTATTCAACATTTCCG	847	lee et al., 2007 ⁴⁴
	Reverse	GTCACAGTTACCAATGCTTA		
<i>bla</i> SHV	Forward	GATGAACGCTTTCCCATGATG	214	Pai et al., 2004 ⁴⁵
	Reverse	CGCTGTTATCGCTCATGGTAA		
<i>bla</i> CTX-M	Forward	GTTACAATGTGAGAAGAG	1018	lee et al., 2007 ⁴⁴
	Reverse	CCGTTTCCGCTATTACAAAC		

According to Paterson et al.¹⁷, ESBL producers are inherently resistant to all cephalosporins, even if they exhibit an *in vitro* susceptibility. In the current investigation, the percentage of ESBL-producing *E. coli* from all-isolated samples was 6 (30%), while the percentage of non-ESBL-producing *E. coli* was 14 (70%). In fact, people in hospitals all across the world are said to struggle with ESBLs. Additionally, it has been noted that the prevalence rates of ESBLs among clinical isolates vary widely across the globe and that these rates rapidly fluctuate over time¹⁸. It is imperative to develop laboratory testing methods to properly

determine the presence of such enzymes in clinical isolates, given the rising incidence of *Enterobacteriaceae* that produce ESBLs¹⁹. Modified double disc synergy tests were the most sensitive of all the ESBL detection techniques²⁰. Similar results were found in a study by Khan et al.²¹, which showed that 6/20 isolates had positive MDDST results and that 6/20 isolates had positive double disk synergy test (DDST) results. Thirty percent of *E. coli* isolates were screened for ESBL production by adhering to the MDDST screening criteria. The presence of one or more ESBL genes in every isolate that passed screening indicated that *E. coli* isolates that produce ESBL are quite

common in the area that is being studied. Kaur *et al.*²², found that 63.4% of *E. coli* isolates from India produced ESBL.

The subtypes of ESBL cannot be identified by phenotypic testing, which only validate the production of ESBL. Nüesch-Inderbinen *et al.*²³, observed that although molecular approaches have been shown to be sensitive, they are expensive, time-consuming, and require specialist equipment. Only molecular detection techniques are likely to lead to the final identification. According to a research by Navon-Venezia *et al.*²⁴, the performance of these phenotypic tests must be regularly assessed because the introduction of new enzymes may alter it. Grover *et al.*²⁵, reported that PCR is a dependable technique for ESBL identification in their investigation of phenotypic and genotypic ESBL detection strategies. The multiplex PCR amplification assay was used in this study to identify the *bla*CTX-M, *bla*SHV, and *bla*TEM genes in the *E. coli* clinical isolates that were recovered. This assay has the advantage of allowing for the quick screening of a large number of clinical isolates, and it can also be used to isolate DNA for use in subsequent molecular epidemiological studies if necessary¹². Furthermore, a trustworthy epidemiological study into antibiotic resistance requires the identification of beta-lactamase. In the current investigation, *E. Coli* isolates taken from the bloodstream of ICU patients clinically suspected of having blood sptecimia in Sana'a, Yemen, were surveyed for antimicrobial drug resistance, ESBL phenotypes, and the identification of *bla*SHV, *bla*TEM, and *bla*CTX-M genes.

Compared to SHV and TEM ESBLs, CTX-M-type ESBLs proved to be the most prevalent kind of ESBL worldwide, with a greater prevalence in most locales^{26,27}. The study revealed that out of the three ESBL genotypes examined, *bla*TEM 6/6 (100%) and *bla* CTX-M 2/6 (33.3%) were the most commonly occurring in *E. coli* isolates that produced ESBL. The *bla*SHV was the least common ESBL genotype, with a prevalence rate of 0% in ESBL-producing *E. coli* isolates. Similar results were found in studies carried out in Iraq by Manoharan *et al.*²⁸, and Pishtiwan and Khadija²⁶. However, TEM ESBL was the most common genotype in our investigation, whereas *bla*SHV-type ESBL was less common. Since just a small sample size of strains from Sana'a city were gathered and analyzed for this study, it is considered that geographical variances are the cause of the discrepancy.

Moreover, earlier research conducted in Yemen has revealed that *E. coli* resistance to third-generation cephalosporins is common there²⁹⁻³⁸. Furthermore, the outbreak reached 50% in Egypt and Syria³⁹, over 70% in Iraq²⁶, and so forth. Moreover, 5-15% of participants in our sample showed susceptibility to all cephalosporins; this incidence is higher than that found in other Arabian countries^{26,28,39}. The study revealed that of the three ESBL genotypes examined, *bla*TEM [6/6 (100%)] and CTX-M [2/6 (33.3%)] were the most commonly occurring in *E. coli* isolates that produced ESBL. Naturally, based on the aforementioned findings, a number of investigations by Teawtrakul *et*

*al.*⁴⁰, Girmenia *et al.*⁴¹, Ricciardi *et al.*⁴², and Devrim *et al.*⁴³, have demonstrated that different nations have different rates and types of *Escherichia* strains identified. These results demonstrate the need for organized national programs in the area that focus on antimicrobial supervision and infection prevention.

Limitations of the study

The following were the study's limitations. Initially, we were unable to precisely identify the kinds of isolates and their pattern of antibiotic sensitivity for Yemen since the data only came from one place (Sana'a city). Confirming the true prevalence of bacterial resistance genes requires molecular study on a large sample size of isolates.

CONCLUSIONS

Knowledge of the antimicrobial resistance patterns and resistance genes of bacterial pathogens in a geographical area is important for control and surveillance of antibiotic resistance. The results of the present study revealed that MDR was highly prevalent. In addition, the carbapenems and amino glycosides were found to be the most active antimicrobial agents *in vitro*. Based on the results obtained in the present study, TEM was highly prevalent among other types of ESBLs genes.

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CONFLICT OF INTEREST

This work does not include any conflicts of interest.

AUTHOR'S CONTRIBUTIONS

Al-Tahish GAA: writing original draft, methodology, investigation. **Al-Yosaffi EA:** conducted the fieldwork as part of his PhD studies. **Othman AM:** writing, review and editing, methodology. **Al-Shamahy HA:** formal analysis, supervision. **Khan M:** writing, review, and editing. **Al-Haddad AM:** writing, review, and editing, data curation. **Al-Moyed KA:** writing, review and editing. **Al-Shawkany ARM:** formal analysis, writing, review, and editing. All authors revised the article and approved the final version.

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