Distribution of *bla*OXA, *bla*SHV, and *bla*TEM type extended-spectrum β-lactamase genes in multi-drug resistant Gram-negative isolates from burn patients of Lahore, Pakistan

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Research

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Abstract

Background Multidrug resistant Gram-negative bacterial pathogens are becoming a lethal source of infections and associated complications of sepsis and multiple organ failure following burn injuries. Genotypic variants of bla OXA , bla SHV and bla TEM type extended-spectrum-β-lactamases (ESBLs) have been detected in Pseudomonas aeruginosa , Enterobacteriaceae and other type of bacteria. These hydrolytic enzymes are responsible for the degradation of broad-spectrum antimicrobials including third generation cephalosporins. We aimed to determine the distribution of ESBLs gene variants among MDR pathogens from post burn infections.

Methods A total of 358 specimens were collected during the period from 15 th August 2017 to 15 th August 2018 from burnt patients at Jinnah Burn and Reconstructive Surgery Centre (JB&RSC).

Results 53.57% cephalosporins resistant isolates were found to be associated with a slightly higher frequency of 50.7% community-acquired and 49.30% nosocomial infections. 72% of these infections was found to be associated with males (p-value = 0.919, OR = 1.038). The age of burn victims ranged from 4 to 85 years (Mean=28.95, SD=±15.65). Pseudomonas spp., were the predominant as 49.33% followed by 22.67% Klebsiella spp., 20% Acinetobacter spp., and 8% Proteus spp., strains. There were 83.33% multidrug resistant isolates and meropenem, imipenem, and amikacin were found to be effective against 28.70%, 25.30%, and 26.00% of cephalosporins resistant strains respectively. Lowest sensitivity of phenotypic tests was observed as 16% ESBLs were detected by double disk synergism test (DDST) and 14% were confirmed by combination disk test (CDT). Molecular detection proved to be effective for the detection of 79.71% bla TEM , 37.68% bla OXA , and 18.84% bla SHV isolates. bla TEM genes were confirmed in 18.18% CDT positive isolates with 62.67% diagnostic accuracy (95% CI = 54.70, 70.00) and 88.42% specificity (95% CI = 80.45, 93.41). All of the bla TEM positive isolates were resistant to cefuroxime and 98.18% were resistant to cephradine, and piperacillin.

Conclusion The antimicrobial resistance associated with the ESBLs producing Pseudomonas spp., and Enterobacteriaceae is becoming a challenge for the treatment and survival of burn patients. The higher frequency of MDR isolates and detection of bla TEM , bla OXA , and bla SHV genes confirms that management of burn patients should be improved to prevent the infections.

Introduction

Burn incidents are frequently reported from low and middle income countries(1). According to WHO report of 2012, worldwide 195000 are caused by burns(2). WHO also reported 7.1 million fire incidents in 2004 and the incidence rate was 110/10000 cases worldwide. Southeast Asia and Middle-East regions were found to be more affected with 243/10000 and 187/10000 incidence respectively, as compared to lowest incidence of 19/10000 in the United States of America(3). Post-burn infections pose a global threat as a major public health problem(4). Nosocomial infections are predominant in burn patients and 75% of deaths occur within a few days of burn exposure due to sepsis and severity of infection (5).

Multidrug resistant Gram-negative bacterial strains are rapidly emerging as etiological agents in 50% of post-burn infections (6). Sepsis is the ultimate consequence of infections caused by bacterial invasion of traumatized skin (7). Both the Gram-negative and positive bacterial strains are reported to be associated with the post burn infections including Pseudomonas spp., Acinetobacter spp., Enterobacteriaceae, Staphylococcus spp., and Streptococcuspyogenes(8). Pseudomonasaeruginosa is the predominant bacterial pathogen among clinical isolates of burnt patients (9).

Multidrug resistance in Gram-negative isolates is found to be associated with the acquisition of β-lactamase gene variants (10). β-lactamases are either encoded in the plasmids or chromosomal DNA (11). ESBLs are known for hydrolyzing the penicillins, third and fourth generation cephalosporins and monobactams (12). Ambler classification involves four classes of β-lactamases including class A, B, C, and D (13). Class A ESBLs such as sulfhydryl variant (SHV) and TemorinaEscherichia coli mutant (TEM) are inactivated by β-lactamase inhibitors including clavulanic acid, sulbactam, tazobactam (14).

Particular types of ESBLs are also capable of inactivating the aminoglycosides and sulphonamides (15). Class D oxacillinases (OXA type ESBLs) including bla_{OXA-10} and bla_{OXA-48} are capable of degrading the cephalosporins and carbapenems respectively (16). Cephamycins and carbapenems are resistant to degradation by ESBLs (13).

To date, 193 variants of bla_{SHV} and 223 variants of bla_{TEM} have been reported worldwide (12). SHV enzymes are commonly found in Enterobacteriaceae including Klebsiella spp., and E. coli but other species also produce them includingP. aeruginosa and Acinetobacterbaumannii(17). bla_{SHV5} and bla_{SHV12} from Korea and Japan, bla_{TEM12} and $bla_{TEM52}$from the United Kingdom, bla_{OXA10} and bla_{OXA13} have been reported from Iran and France respectively (18). A recent study from Pakistan revealed 40% of ESBLs producing bacteria in burn isolates (19). Horizontal transfer by plasmids and transposons during conjugation is a principal genetic factor for worldwide

dissemination of ESBLs encoding genes (20). Self-medication is a contributing factor behind rapidly developing antimicrobial resistance (21). Rapid and accurate diagnosis of infectious agents is necessary for appropriate antibiotic prescription (22). The main objective of this study was to determine the frequency distribution of MDR bacterial pathogens implicated in post-burn infections. Secondly, we aimed to determine the frequency of the most prevalent types of genetic variants of bla_{OXA}, bla_{SHV}, and bla_{TEM} ESBLs encoding genes that might be associated with the dissemination of antimicrobial resistance in the community acquired and nosocomial pathogens. The molecular detection of ESBLs by multiplex PCR was employed in order to test the validatetheir use as a routine diagnostic procedure which would reduce the cost and duration of the treatment.

Methods

Study design and clinical setting

A cross-sectional study was conducted at Jinnah Burn and Reconstructive Surgery Centre (JB&RSC) and the Department of Microbiology and Molecular Genetics, University of the Punjab Lahore. Ethical approval was obtained from Jinnah Hospital Lahore on 40th meeting of the Ethical Review Board on 12th August 2017. The bacteriological profiling, antimicrobial susceptibility testing (AST), and genotypic screening were performed during the period from 15th August 2017 to 15th August 2018. Both pediatric and adult patients were included for the analysis if they had clinical signs and symptoms such as pain, swelling of burn wounds and infection. The cephalosporins and carbapenems resistant Gram-negative isolates were further processed for the phenotypic identification and genetic profiling of ESBLs by *bla_{OXA}*, *bla_{SHV}*, and *bla_{TEM}* multiplex PCR. Burnt patients suffering from previous infections,those receiving antibiotic therapy, and cephalosporins sensitive Gram-negative and positive isolates were excluded.

Bacteriological profiling and data collection

A total of 358 samples including wounds swabs, blood and tissue biopsy specimens were collected from the patients under treatment in burn unit's OPD, general ward, intensive care unit (ICU), and plastic surgery ward. Gram-positive isolates were excluded from further analysis.Antimicrobial susceptibility testing was performed after identification of bacterial isolates.

Antimicrobial susceptibility testing and phenotypic detection of ESBLs

The antimicrobial resistance and susceptibility patterns were analyzed by performing Kirby Bauer's disk diffusion method according to Clinical Laboratory Standards Institute (CLSI) 2017 guidelines[23].Bioanalyse®(Turkey) antimicrobial discs were used for AST profiling of Gram-negative bacterial isolates.Preliminary ESBLs detection was performed by double disk synergism test (DDST) and confirmatory combination disk test (CDT) (24).

bla_{OXA}, *bla_{SHV}*, and *bla_{TEM}* multiplex PCR

Whole-genomic DNA extraction was performed by the boiling lysis method by preparing the cells suspension of purely isolated bacterial colonies as described previously (25). Previously designed conserved regions specific *bla_{OXA}*, *bla_{SHV}*, and *bla_{TEM}*primers were optimized for multiplex PCR (26,27). PCR amplicons were visualized by agarose-gel electrophoresis with 1% agarose gel and 1X Tris-borate-EDTA (TBE) buffer.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 23 has been used for the execution of all statistical analyses. The association of ESBLs genotypes with the antimicrobial resistance has been demonstrated by percentages. The p-value of < 0.05 has been taken as significant in the frequency distribution of infections among males and females and validity testing of ESBLs genes detection.

Results

Distribution of clinical isolates

During one year, n=358 specimens were collected from burns patients admitted in Jinnah Burn and Reconstructive Surgery Centre (JB&RSC) Lahore. Among n=280(78.21%) positive cultures, n=150(53.57%) cephalosporins resistant isolates were further processed for the detection of ESBLs by phenotypic tests and multiplex PCR. Age of the patients ranged from minimum of 4 years to maximum of 85 years (Mean=28.95, SD=±15.65).

The majority of cases n=106(70.67%) were reported as inpatients and n=44(29.33%) as outdoor patients. *Pseudomonas* spp., was the most commonly isolated pathogen in n=74(49.33%) patients while *Klebsiella* spp., infected 34(22.67%) patients. *Acinetobacter* spp., and *Proteus* spp., were isolated from n=30(20.00%), and n=12(8.00%) patients respectively.

Antimicrobial susceptibility testing and phenotypic detection of ESBLs

Meropenem and imipenem proved to be effective only against 43(28.70%) and 38(25.30%) isolates respectively. Cefoperazone amongst cephalosporins demonstrated sensitivity against 22(14.70%) strains followed by cefotaxime 14(9.30%) and ceftazidime 14(9.30%). Amikacin was the most effective aminoglycoside with 39(26.00%) of isolates being sensitive. Aztreonam and tigecycline exhibited the intermediate sensitivity patterns in 36(24.00%) and 21(14.00%) isolates. Polymyxin E with 83(55.30%) sensitivity proved to bethe most effective therapeutic agent for post-burn infections. There were 125(83.33%) cephalosporins resistant MDR isolates with resistance against more than two or three antimicrobial agents. These multidrug resistant strains were also resistant to nalidixic acid and tetracycline (Table-1).There were 24(16.00%) ESBLs producing cephalosporins resistant isolates detected by double disk synergism test (DDST) of which 21(14.00%) were also detected by confirmatory combination disk test (CDT). *Pseudomonas*spp., was the predominant ESBLs producer as 10(13.51%) strains were confirmed by CDT as ESBLs producers. *Klebsiella* spp., *Acinetobacter* spp., and *Proteus* spp., strains were relatively low in number with 5(14.71%), 4(13.33%), and 2(16.67%) as ESBLs producers respectively. A large number of these cephalosporins resistant isolates were not-determined phenotypically as ESBLs producers.

Detection of bla_{OXA}, bla_{SHV}, and bla_{TEM} type ESBLs genes

All of the cephalosporins resistant strains were screened by PCR for the detection of ESBLs encoding genes. There were 69(46.00%) isolates found to be positive for ESBLs genes as detected by PCR. ESBLs genes were detected in 29(39.19%) *Pseudomonas* spp., 23(67.64%) *Klebsiella* spp., 13(43.33%) *Acinetobacter* spp., and 4(33.33%) *Proteus* spp., strains. Multiplex PCR confirmed co-existence of bla_{TEM} and bla_{SHV} genes in 12(17.39%) patients. bla_{TEM} and bla_{OXA} genes co-existence was found in 10(14.49%) of patients. bla_{OXA}, $bla_{SHV}$and bla_{TEM} co-existence was observed in only 4(5.79%) of patients. bla_{TEM} genes were detected in 55(79.71%) isolates with the highest frequency of 21(91.30%) in *Klebsiella* spp., isolates. bla_{OXA}, bla_{SHV}, and bla_{TEM} co-existence was found in three *Klebsiella* spp., isolates but only one *Proteus* spp., isolates (Table-2).

Validity analysis

bla_{TEM} genes were reported in 11(20.00%) of DDST positive isolates with 62.00% diagnostic accuracy (95% CI = 54.02, 69.38) and 86.32% specificity (77.98, 91.83) as shown in Table-3A. These bla_{TEM}genes were also confirmed in 10(18.18%) CDT positive isolates with 62.67% diagnostic accuracy (95% CI = 54.70, 70.00) and 88.42% specificity (95% CI = 80.45, 93.41) as shown in Table-3B.

Association of ESBLs genes with antimicrobial resistance

All of the bla_{TEM} positive isolates were resistant to cefuroxime and 54(98.18%) were resistant to cephradine, piperacillin, gentamicin, and tetracycline. 35(63.64%) bla_{TEM} positive isolates were resistant to meropenem and 27(49.09%) were resistant to polymyxin E. All ofbla_{OXA} positive isolates 26(100%) were resistant to amoxicillin-clavulanate and 25(96.15%) were resistant to cephradine, cefuroxime, doripenem, ertapenem, ciprofloxacin and tetracycline. All of the bla_{SHV} positive isolates 13(100%) were resistant to piperacillin and cefuroxime (Table-4).

Discussion

This study includes assessment of cephalosporins and carbapenems resistance in burns patients' clinical isolates. The frequencies of ESBLs producing bacteria also have been determined in order to find the association with antimicrobial resistance patterns. There was some small difference between the community-acquired (50.70%) and nosocomial infections (49.30%). Community acquired infections were less prevalent about a decade ago where only 16.90% previously infected burn patients were hospitalized (28). These findings indicate that the MDR strains are currently proliferating in the environment and the community. Self-prescription and the ease of access to the commercially available antibiotics and inappropriate prescriptions by physicians may be the contributing factors in the emergence of MDR strains (13,18).Individuals with the younger age work in different factories and industries. Most of the burn victims belonged to the young age of 20–30 years in accordance with earlier research (29).

Pseudomonas spp., is the leading causative agent of burn wound infections and causes sepsis mediated mortality in 40–50% cases (30). All of these pathogens especially Pseudomonas spp., and Klebsiella spp., are capable of adhering with and forming biofilms on inanimate objects such as catheters and surgical instruments (31). Here, the single bacterial strains were processed instead of multiple isolates for the

antimicrobial susceptibility testing in order to determine the frequency of MDR Gram-negative pathogens. Previously multiple bacterial strains have been isolated from the burn'spatients with Pseudomonas spp., and Acinetobacter spp.,co-infection (32).

There were 83.33% (125/150) MDR isolates showing resistance against three and more classes of antimicrobial agents. These isolates were observed with more than 70% resistance against meropenem and imipenem. Early investigations on burns patients differ where more than 80% isolates were resistant to imipenem and meropenem as all of the isolates were included in the analyses (33). Molecular detection by multiplex PCR was useful as 46% of cephalosporins resistant isolates were positive for ESBLs genes. The remaining 54% resistant isolates may harbor metallo-β-lactamases (MBLs) encoding genes and other non-enzymatic resistance mechanisms. Several phenotypically negative isolates were identified by multiplex PCR as ESBLs producers. Low specificity and lack of constant sensitivity of the phenotypic tests justifies the need to use more advance molecular techniques for the rapid, specific and accurate diagnosis of ESBLs producers (34).

bla $_{TEM}$was predominant in cephalosporins resistant isolates followed by bla$_{OXA}$ and bla$_{SHV}$. These findings are in agreement with Bajpai et al., form New Delhi, India where bla$_{TEM}$was detected in 48.70% isolates followed by bla$_{SHV}$(35). Shakibaieet al., reported 6.6% bla$_{SHV}$and of 2.5% bla$_{TEM}$from burn patients in Iran (30). The differences in the occurrence of ESBLs genes are based on their geographical distribution as bla$_{TEM}$is predominant in China and bla$_{SHV}$is the leading ESBL in North-America (12). Co-existence of ESBLs genes was confirmed in our findings where bla$_{SHV}$ and bla$_{TEM}$ was detected in 17.39%, bla$_{OXA}$ and bla$_{TEM}$ in 14.49 PCR positive isolates. These findings differ from Parajuli et al., where bla$_{SHV}$ and bla$_{TEM}$co-existed in 10% isolates(5). bla$_{OXA}$, bla$_{SHV}$, and bla$_{TEM}$ co-existence was detected in 5.78% of isolates which indicates the parallel emergence of different ESBLs in the same isolates from burns patients and their surroundings.

The clinical presentation of patients suffering from post-burn infections is very difficult to interpret which can lead to the inappropriate prescription of antibiotics. Multi-drug resistance in burn isolates develops due to slow wound healing associated hospitalizations poor diagnosis and treatment. The incidence of post-burn infections by multi-drug resistant bacterial pathogens urgently needs to be accessed.Bacteriological and genetic profiling of ESBLs and other resistance factors provides guidance for the empiric therapy and ensures proper diagnosis.The use of multiplex PCR is very reliable and it ensures cost-effectiveness and speedy output as compared to traditional culturing and phenotypic tests. Multiplex PCR can be employed in infection control programs and to ensure higher throughput in case of outbreaks.

Conclusions

Multidrug resistant Gram-negative bacterial pathogens are responsible for the spread of antimicrobial resistance in our community and healthcare settings. Emergence of Pseudomonas spp., as the predominant pathogens followed by Enterobacteriaceae is becoming problematic in the treatment of burns patients. Phenotypic testing of ESBLs is less effective due to lower sensitivity of DDST and CDT. Molecular detection of ESBLs encoding genes by multiplex PCR is more accurate, reliable, and specific diagnostic method. bla$_{TEM}$ followed by bla$_{OXA}$ ESBLs are frequently associated with the inefficacy of third generation cephalosporins and carbapenems. bla$_{SHV}$ ESBLs were fewer in number but all of these isolated were resistant to piperacillin. It is necessary to overcome the emergence of multidrug resistant bacterial pathogens by understanding the antimicrobial resistance mechanisms other than enzymatic degradation of antibiotics. The spread of multidrug resistant strains in burn clinical settings is alarming therefore treatment strategy and infection-control management should be improved immediately.

Abbreviations

CLSI:Clinical Laboratory Standards Institute; bla$_{OXA}$:Oxacillinases; bla$_{SHV}$:β-lactamase sulfhydryl variant; bla$_{TEM}$:β-lactamase TemorinaEscherichia coli mutant; ERB; Ethical Review Board; CDST:Combination disc test; DDST:Double disc synergy test; ESBL:Extended-spectrum β-Lactamase; JB&RSC:Jinnah Burn Unit and Reconstructive Surgery Centre; MDR:Multidrug-resistant; MMG:Department of Microbiology and Molecular Genetics.

Declarations Section

Ethical approval and consent to participate

This study has been approved by the Ethical Review Board (ERB) of Allama Iqbal Medical College (AIMC) & Jinnah Hospital Lahore in its 40th meeting held on 12th August, 2017.

Consent for publication

Not applicable

Availability of data and materials

The data sets analyzed during the current study are available from the corresponding author.

Competing interests

Authors declare that they have no competing interests.

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Declaration

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Author's contribution

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Tables

Table-1: Antimicrobial sensitivity and resistance patterns of clinical isolates from burnt patients.

Antibiotics	Antimicrobial susceptibility patterns of isolates		
	Sensitive (S) n (%)	Intermediate (I) n (%)	Resistant (R) n (%)
Penicillins / β-lactamase inhibitors			
Piperacillin (PIP)	7 (4.70%)	2 (1.30%)	141 (94.00%)
Amoxycillin-clavulanate (AMC)	8 (5.30%)	2 (1.30%)	140 (93.30%)
Ampicillin-sulbactam (SAM)	13 (8.70%)	6 (4.00%)	131 (87.30%)
Piperacillin-tazobactam (TZP)	26 (17.30%)	14 (9.30%)	110 (73.30%)
Cephalosporins / β-lactamase inhibitors			
Ceftazidime (CAZ)	14 (9.30%)	14 (9.30%)	122 (81.30%)
Cephradine (CE)	2 (1.30%)	1 (0.70%)	147 (98.00%)
Cefoperazone (CFP)	22 (14.70%)	5 (3.30%)	123 (82.00%)
Cefoperazone-sulbactam (SCF)	38 (25.30%)	10 (6.70%)	102 (68.00%)
Cefotaxime (CTX)	14 (9.30%)	5 (3.30%)	131(87.30%)
Ceftriaxone (CRO)	12 (8.00%)	6 (4.00%)	132 (88.00%)
Cefuroxime (CXM)	2 (1.30%)	2 (1.30%)	146 (97.30%)
Carbapenems			
Doripenem (DOR)	17 (11.30%)	-	133 (88.70%)
Ertapenem (ETP)	5 (3.30%)	-	145 (96.70%)
Imipenem (IMI)	38 (25.30%)	9 (6.00%)	103 (68.70%)
Meropenem (MEM)	43 (28.70%)	2 (1.30%)	105 (70.00%)
Aminoglycosides			
Amikacin (AK)	39 (26%)	7 (4.70%)	104 (69.30%)
Gentamicin (GEN)	8 (5.30%)	-	142 (94.70%)
Tobramycin (TOB)	7 (4.70%)	-	143 (95.30%)
Quinolones / Fluoroquinolones			
Nalidixic acid (NAL)	6 (4.00%)	-	144 (96.00%)
Ciprofloxacin (CIP)	11 (7.30%)	-	139 (92.70%)
Levofloxacin (LEV)	18 (12.00%)	3 (2.00%)	129 (86.00%)
Ofloxacin (OFL)	5 (3.30%)	3 (2.00%)	142 (94.70%)
Others			
Aztreonam (ATM)	13 (8.70%)	36 (24.00%)	101 (67.30%)
Tigecycline (TGC)	17 (11.30%)	21 (14.00%)	112 (74.70%)
Tetracycline (TET)	4 (2.70%)	-	146 (97.30%)
Polymyxin E (PE)	83 (55.30%)	-	67 (44.70%)

Table-2: ESBLs genotypes found to be disseminated among PCR positive clinical isolates.

PCR positive isolates	ESBLs genotypes						
	bla$_{OXA}$ n (%)	*bla*$_{SHV}$ n (%)	*bla*$_{TEM}$ n (%)	*bla*$_{OXA}$-*bla*$_{SHV}$ n (%)	*bla*$_{SHV}$-*bla*$_{TEM}$ n (%)	*bla*$_{OXA}$-*bla*$_{TEM}$ n (%)	*bla*$_{OXA}$-*bla*$_{SHV}$-*bla*$_{TEM}$ n (%)
Pseudomonas spp., 29 (42.03%)	14 (48.28%)	2 (6.89%)	18 (62.07%)	-	2 (6.89%)	3 (10.34%)	-
Klebsiella spp., 23 (33.33%)	6 (26.09%)	9 (39.13%)	21 (91.30%)	3 (13.04%)	8 (34.78%)	2 (8.69%)	3 (13.04%)
Acinetobacter spp., 13 (18.84%)	4 (30.77%)	1 (7.69%)	12 (92.31%)	-	1 (7.69%)	5 (38.46%)	-
Proteus spp., 4 (5.78%)	2 (50.00%)	1 (25.00%)	4 (100%)	1 (25.00%)	1 (25.00%)	-	1 (25.00%)
Total (n=69)	**26 (37.68%)**	**13 (18.84%)**	**55 (79.71%)**	**4 (5.78%)**	**12 (17.39%)**	**10 (14.49%)**	**4 (5.78%)**

Table-3: Validity testing of ESBLs detection by phenotypic testing and molecular detection by multiplex PCR.

Table-3A: Validity analysis of ESBLs detection by DDST as compared to multiplex PCR.

Multiplex PCR	DDST Positive n (%)	DDST Not-determined n (%)	DDST Sensitivity (%), 95% CI	DDST Specificity (%), 95% CI	DDST Diagnostic Accuracy (%), 95% CI	Positive DDST Predictive Value (%), 95% CI	Negative DDST Predictive Value (%), 95% CI	Positive DDST Likelihood Ratio, 95% CI	Negative DDST Likelihood Ratio, 95% CI	$\chi 2$	p-value
bla$_{OXA}$Positive	3 (13.04%)	23 (88.46%)	11.54% (4.003, 28.98)	83.06% (75.49, 88.65)	70.67% (62.94, 77.36)	12.50% (4.344, 31)	81.75% (74.1, 87.52)	0.6813 (0.004145 - 112)	1.065 (0.9742 - 1.164)	0.466	0.769
bla$_{OXA}$Negative	21 (16.94%)	103 (83.06%)									
bla$_{SHV}$Positive	3 (23.07%)	10 (76.92%)	23.08% (8.179, 50.26)	84.67% (77.7, 89.75)	79.33% (72.16, 85.04)	12.50% (4.344, 31)	92.06% (86.01, 95.63)	1.505 (0.1554 - 14.59)	0.9085 (0.7445 - 1.109)	0.530	0.438
bla$_{SHV}$Negative	21 (15.32%)	116 (84.67%)									
bla$_{TEM}$Positive	11 (20%)	44 (80 %)	20% (11.55, 32.37)	86.32% (77.98, 91.83)	62% (54.02, 69.38)	45.83% (27.89, 64.93)	65.08% (56.42, 72.85)	1.462 (0.6163 - 3.466)	0.9268 (0.8831 - 0.9727)	1.034	0.309
bla$_{TEM}$Negative	13 (13.68%)	82 (86.34%)									

Table-3B: Validity analysis of ESBLs detection by CDT as compared to multiplex PCR.

Multiplex PCR	CDT Positive n (%)	CDT Not-determined n (%)	CDT Sensitivity (%), 95% CI	CDT Specificity (%), 95% CI	CDT Diagnostic Accuracy (%), 95% CI	Positive CDT Predictive Value (%), 95% CI	Negative CDT Predictive Value (%), 95% CI	Positive CDT Likelihood Ratio, 95% CI	Negative CDT Likelihood Ratio, 95% CI	$\chi 2$	p-value
bla$_{OXA}$Positive	5 (19.23%)	21 (80.77%)	19.23% (8.507, 37.88)	87.10% (80.06, 91.9)	75.33% (67.86, 81.54)	23.81% (10.63, 45.09)	83.72% (76.39, 89.1)	1.49 (0.2541 - 8.74)	0.9274 (0.8424 - 1.021)	0.715	0.368
bla$_{OXA}$Negative	16 (12.90%)	108 (87.09%)									
bla$_{SHV}$Positive	2 (15.38%)	11 (84.62%)	15.38% (4.326, 42.24)	86.13% (79.35, 90.94)	80% (72.89, 85.62)	9.524% (2.652, 28.91)	91.47% (85.38, 95.17)	1.109 (0.004565 - 269.6)	0.9824 (0.8199 - 1.177)	0.023	1.000
bla$_{SHV}$Negative	19 (13.87%)	118 (86.13%)									
bla$_{TEM}$Positive	10 (18.18%)	45 (81.82%)	18.18% (10.19, 30.33)	88.42% (80.45, 93.41)	62.67% (54.7, 70)	47.62% (28.34, 67.63)	65.12% (56.56, 72.8)	1.57 (0.5439 - 4.533)	0.9253 (0.8832 - 0.9695)	1.261	0.261
bla$_{TEM}$Negative	11 (11.58%)	84 (88.42%)									

Table-4: Association of ESBLs genes with the antimicrobial susceptibility patterns of clinical isolates.

Antibiotics	ESBLs genes detected by multiplex PCR n/150 (%)								
	bla$_{OXA}$ 26/150 (17.30%)			*bla*$_{SHV}$ 13/150 (8.67%)			*bla*$_{TEM}$ 55/150 (36.67%)		
	S n (%)	I n (%)	R n (%)	S n (%)	I n (%)	R n (%)	S n (%)	I n (%)	R n (%)
PIP	3 (11.54%)	-	23 (88.46%)	-	-	13 (100%)	-	1 (1.82%)	54 (98.18%)
AMC	-	-	26 (100%)	1 (7.69%)	-	12 (92.31%)	4 (7.27%)	-	51 (92.73%)
SAM	2 (7.69%)	-	24 (92.31%)	1 (7.69%)	-	12 (92.31%)	5 (9.09%)	1 (1.82%)	49 (89.09%)
TZP	5 (19.23%)	3 (11.54%)	18 (69.23%)	2 (15.38%)	3 (23.08%)	8 (61.54%)	11 (20.00%)	6 (10.91%)	38 (69.09%)
CAZ	1 (3.85%)	4 (15.38%)	21 (80.77%)	1 (7.69%)	1 (7.69%)	11 (84.62%)	2 (3.64%)	6 (10.91%)	47 (85.45%)
CE	-	1 (3.85%)	25 (96.15%)	-	1 (7.69%)	12 (92.31%)	-	1 (1.82%)	54 (98.18%)
CFP	3 (11.54%)	-	23 (88.46%)	3 (23.08%)	-	10 (76.92%)	5 (9.09%)	3 (5.45%)	47 (85.45%)
SCF	6 (23.08%)	4 (15.38%)	16 (61.54%)	3 (23.08%)	2 (15.38%)	8 (61.54%)	15 (27.27%)	6 (10.91%)	34 (61.82%)
CTX	3 (11.54%)	1 (3.85%)	22 (84.62%)	-	1 (7.69%)	12 (92.31%)	3 (5.45%)	3 (5.45%)	49 (89.09%)
CRO	1 (3.85%)	2 (7.69%)	23 (88.46%)	1 (7.69%)	2 (15.38%)	10 (76.92%)	4 (7.27%)	3 (5.45%)	48 (87.27%)
CXM	-	1 (3.85%)	25 (96.15%)	-	-	13 (100%)	-	-	55 (100%)
DOR	1 (3.85%)	-	25 (96.15%)	5 (38.46%)	-	8 (61.54%)	11 (20.00%)	-	44 (80.00%)
ETP	1 (3.85%)	-	25 (96.15%)	1(7.69%)	-	12(92.31%)	2 (3.64%)	-	53 (96.36%)
IMI	7 (26.92%)	1 (3.85%)	18 (69.23%)	4 (30.77%)	1 (7.69%)	8 (61.54%)	17 (30.91%)	4 (7.27%)	34 (61.82%)
MEM	9 (34.62%)	-	17 (65.38%)	5 (38.46%)	-	8 (61.54%)	20 (36.36%)	-	35 (63.64%)
AK	6 (23.08%)	2 (7.69%)	18 (69.23%)	4 (30.77%)	-	9 (69.23%)	17 (30.91%)	1 (1.82%)	37 (67.27%)
GEN	2 (7.69%)	-	24 (92.31%)	1 (7.69%)	-	12 (92.31%)	1 (1.82%)	-	54 (98.18%)
TOB	2 (7.69%)	-	24 (92.31%)	-	-	13 (100%)	2 (3.64%)	-	53 (96.36%)
NAL	2 (7.69%)	-	24 (92.31%)	1 (7.69%)	-	12 (92.31%)	4 (7.27%)	-	51 (92.73%)
CIP	1 (3.85%)	-	25 (96.15%)	1 (7.69%)	-	12 (92.31%)	3 (5.45%)	-	52 (94.55%)
LEV	3 (11.54%)	-	23 (88.46%)	1 (7.69%)	-	12 (92.31%)	8 (14.55%)	1 (1.82%)	46 (83.64%)
OFL	1 (3.85%)	1 (3.85%)	24 (92.31%)	1 (7.69%)	1 (7.69%)	11 (84.62%)	1 (1.82%)	1 (1.82%)	53 (96.36%)
ATM	1 (3.85%)	11	14 (53.85%)	-	4 (7.27%)	9 (69.23%)	1 (1.82%)	11 (20.00%)	43 (78.18%)
TGC	4 (15.38%)	3 (11.54%)	19 (73.08)	4 (30.77%)	2 (15.38%)	7 (53.85%)	7 (12.73%)	6 (10.91%)	42 (76.36%)
TET	1 (3.85%)	-	25 (96.15%)	-	-	13 (100%)	1 (1.82%)	-	54 (98.18%)
PE	13 (50.00%)	-	13 (50.00%)	7 (53.85%)	-	6 (46.15%)	28 (50.91%)	-	27 (49.09%)