**Original Article**

**bla**\textsubscript{OXA}, **bla**\textsubscript{SHV}, and **bla**\textsubscript{TEM} extended-spectrum \(\beta\)-lactamases in Gram-negative strains from burn patients in Lahore, Pakistan

Muhammad Hayat Haider\textsuperscript{1}, Noor Ul Ain\textsuperscript{1}, Samyyia Abrar\textsuperscript{1}, Saba Riaz\textsuperscript{1,2}

\textsuperscript{1} Department of Microbiology and Molecular Genetics, University of Punjab, Lahore, Pakistan
\textsuperscript{2} Citilab and Research Centre, Lahore, Pakistan

**Abstract**

Introduction: Gram-negative bacterial pathogens are associated with complications of post-burn infections that showed significant association with different genotypic variants of extended-spectrum \(\beta\)-lactamases. In this study, we aimed to determine the distribution of ESBL gene variants among bacterial pathogens from post-burn infections.

Methodology: Cephalosporin-resistant isolates were collected from Jinnah Burn and Reconstructive Surgery Centre, Lahore. Phenotypic testing: double-disk synergy test, combination disk test, multiplex PCR for **bla**\textsubscript{OXA}, **bla**\textsubscript{TEM}, and **bla**\textsubscript{SHV} genes were performed.

Results: Of the 53.5% cephalosporin-resistant isolates, 50.7% were community-acquired and 49.3% were nosocomial pathogens. Seventy-two percent of post-burn infections were found in males (p-value = 0.92, OR = 1.04). The age of burn victims ranged from 4 to 85 years (mean = 28.9, SD = 15.6). \textit{Pseudomonas aeruginosa} was most predominant at a rate of 49%. There were 83.3% multidrug-resistant isolates, which showed susceptibility to meropenem, imipenem, and amikacin in 28.7%, 25.3%, and 26% respectively. In phenotypic tests 16% ESBLs detected by the DDST and 14% confirmed by the CDT. Molecular detection proved effective for the detection of 79.7% **bla**\textsubscript{TEM}, 37.7% **bla**\textsubscript{OXA}, and 18.8% **bla**\textsubscript{SHV} isolates. **bla**\textsubscript{TEM} genes were confirmed in 18.1% CDT-positive isolates, with 62.6% diagnostic accuracy (95% CI = 54.7-70) and 88% specificity (95% CI = 80.4-93.4).

Conclusions: The antimicrobial resistance associated with the ESBL-producing \textit{Pseudomonas} spp. and \textit{Enterobacteriaceae} is becoming a challenge for the treatment and survival of burn patients. The high rate **bla**\textsubscript{TEM}, **bla**\textsubscript{OXA}, and **bla**\textsubscript{SHV} genes confirm the need to improve the management of burn patients in order to prevent post-burn infections.

**Key words:** Burns; ESBLs; **bla**\textsubscript{OXA}; **bla**\textsubscript{SHV}; **bla**\textsubscript{TEM}; diagnostic accuracy.

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**Introduction**

Burn incidents are frequently reported from low and middle income countries [1]. According to WHO report of 2012, worldwide 195,000 deaths happen due to burns [2]. WHO also reported 7.1 million fire incidents in 2004 and the incidence rate was 110/10,000 cases worldwide. Southeast Asia and Middle-East region was found more affected with an incidence rate of 243/10,000 and 187/10,000 respectively, as compared to lowest incidence of 19/10,000 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 \textit{Pseudomonas} spp., \textit{Acinetobacter} spp., \textit{Enterobacteriaceae}, \textit{Staphylococcus} spp., and \textit{Streptococcus pyogenes} [8]. \textit{Pseudomonas aeruginosa} is the predominant bacterial pathogen among clinical isolates of burn patients [9].

To date, 193 variants of **bla**\textsubscript{SHV} and 223 variants of **bla**\textsubscript{TEM} have been reported worldwide [10]. SHV enzymes are commonly found in \textit{Enterobacteriaceae} including \textit{Klebsiella} spp., and \textit{E. coli} but other species also exit including \textit{P. aeruginosa} and \textit{A. baumannii} [11]. **bla**\textsubscript{SHV}5 and **bla**\textsubscript{SHV}12 from Korea and Japan, **bla**\textsubscript{TEM}12 and **bla**\textsubscript{TEM}52 from United Kingdom, **bla**\textsubscript{OXA}10 and **bla**\textsubscript{OXA}13 have been reported from Iran and France respectively [12]. Recent study from Pakistan reveals 40% of ESBLs producing bacteria are detected in burn patients [13]. Horizontal transfer by plasmids and transposons during conjugation is a principal genetic
factor for worldwide dissemination of ESBLs encoding genes [14]. Self-medication is a contributing factor behind rapidly developing antimicrobial resistance [15]. Rapid and accurate diagnosis of infectious agents is necessary for appropriate antibiotic prescription [16]. 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 \textit{bla}\textsubscript{OXA}, \textit{bla}\textsubscript{SHV}, and \textit{bla}\textsubscript{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 validity of phenotypic tests to be involved as routine diagnostic procedure in order to reduce the cost and duration of the treatment.

**Methodology**

**Study design and clinical setting**

A cross-sectional study was conducted at Jinnah Burn and Reconstructive Surgery Centre (JB and RSC), Lahore, Pakistan and the Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore. The burn unit at JB and RSC consists of 75 beds, where sampling was performed on inpatients and outpatients. Ethical approval was obtained from Jinnah Hospital Lahore at the 40th meeting of the Ethical Review Board on 12th August 2017. Bacteriological profiling, antimicrobial susceptibility testing (AST), and genotypic screening were performed between 15th August 2017 and 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 multidrug-resistant Gram-negative isolates were susceptible to at least one antibiotic in three or more antimicrobial classes [6]. The cephalosporin- and carbapenem-resistant Gram-negative isolates were further processed for phenotypic identification and genetic profiling of ESBLs by \textit{bla}\textsubscript{OXA}, \textit{bla}\textsubscript{SHV}, and \textit{bla}\textsubscript{TEM} multiplex PCR. Burn patients suffering from previous infections, those receiving antibiotic therapy, and cephalosporin-sensitive Gram-negative isolates were excluded.

**Bacteriological profiling and data collection**

A total of 358 non-repetitive samples, including wound swabs, blood and tissue biopsy specimens, were collected from the patients undergoing treatment in the outpatient department (OPD), general ward, intensive care unit (ICU), and plastic surgery ward.

Antimicrobial susceptibility testing was performed after identification of bacterial strains.

**Antimicrobial susceptibility testing and phenotypic detection of ESBLs**

Antimicrobial resistance and susceptibility patterns were analyzed by Kirby Bauer’s disk diffusion method, according to the Clinical and Laboratory Standards Institute (CLSI) 2017 guidelines [17]. Antimicrobial discs (Bioanalyse, Ankara, Turkey) including piperacillin, amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam, ceftazidime, cephradine, cefoperazone, cefoperazone-sulbactam, cefotaxime, ceftriaxone, cefuroxime, doripenem, ertapenem, imipenem, meropenem, amikacin, gentamicin, tobramycin, nalidixic acid, ciprofloxacin, levofloxacin, ofloxacin, aztreonam, tigecycline, tetracycline, and polymyxin E were used for AST profiling of Gram-negative bacterial isolates. Preliminary ESBL detection was performed by double-disk synergy tests (DDST) and confirmatory combination disk tests (CDT) [18].

\textit{bla}\textsubscript{OXA}, \textit{bla}\textsubscript{SHV}, and \textit{bla}\textsubscript{TEM} multiplex PCR

Whole-genomic DNA extraction was performed by boiling lysis method, preparing the cell suspension of purely isolated bacterial colonies, as performed previously [19]. Already designed conserved regions, specific \textit{bla}\textsubscript{OXA}, \textit{bla}\textsubscript{SHV}, and \textit{bla}\textsubscript{TEM} primers were optimized for multiplex PCR [20,21]. 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 was used for all statistical analyses. A p-value < 0.05 was considered as significant for the frequency distribution of infections among males and females and the validity testing of ESBL gene detection.

**Results**

**Distribution of clinical isolates**

From August 2017 to August 2018, 358 specimens were collected from burn patients admitted to Jinnah Burn and Reconstructive Surgery Centre (JB and RSC), Lahore. Among 280 (78.2%) positive cultures, 150 (53.5%) cephalosporin-resistant isolates were further processed for the detection of ESBLs by phenotypic tests and multiplex PCR. The patients’ age ranged from a minimum of 4 years to a maximum of 85 years (Mean = 28.9, SD = 15.6).
Of the 150 cephalosporin-resistant isolates, a majority of 106 (70.6%) isolates were collected from inpatients and the remaining 44 (29.3%) from outpatients. *Pseudomonas* spp were the most commonly isolated pathogens, found in 74 (49.3%) patients, while *Klebsiella* spp. infected 34 (22.6%) patients. *Acinetobacter* spp. and *Proteus* spp. were isolated from 30 (20%), and 12 (8%) patients, respectively.

**Antimicrobial susceptibility testing and phenotypic detection of ESBLs**

Meropenem and imipenem proved to be effective only against 43 (28.7%) and 38 (25.3%) isolates respectively. Cefoperazone demonstrated sensitivity to 22 (15%) strains of cephalosporin, followed by cefotaxime and ceftazidime to 14 (9.3%) strains each. Amikacin was the most effective aminoglycoside, proving sensitive to 39 (26%) isolates. Aztreonam and tigecycline exhibited intermediate sensitivity to 36 (24%) and 21 (14%) isolates. Polymyxin E, with its sensitivity to 83 (55.3%) isolates, emerged as the most effective therapeutic agent for post-burn infections. There were 125 (83.3%) cephalosporin-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%) ESBL-producing cephalosporin-resistant isolates detected by DDST, of which CDT also confirmed 21 (14%). *Pseudomonas* spp. was the predominant ESBL producer, as 10 (13.5%) such strains were confirmed by CDT. The number of ESBL producers in *Klebsiella* spp., *Acinetobacter* spp., and *Proteus* spp. strains were relatively low: 5 (15.7%), 4 (13.3%), and 2 (16.6%), respectively. A large number of these cephalosporin-resistant isolates, 126 (84%), were not phenotypically determined as ESBL producers.

**Table 1.** Antimicrobial sensitivity and resistance patterns of clinical isolates from burn patients.

| Antibiotics                          | Sensitive (S) | Intermediate (I) | Resistant (R) |
|--------------------------------------|---------------|------------------|--------------|
| **Penicillins/β-lactamase inhibitors** |               |                  |              |
| Pipericillin (PIP)                   | 7 (4.7%)      | 2 (1.3%)         | 141 (94%)    |
| Amoxycillin-clavulanate (AMC)        | 8 (5.3%)      | 2 (1.3%)         | 140 (93.3%)  |
| Ampicillin-sulbactam (SAM)           | 13 (8.7%)     | 6 (4%)           | 131 (87.3%)  |
| Pipericillin-tazobactam (TZP)        | 26 (17.3%)    | 14 (9.3%)        | 110 (73.3%)  |
| **Cephalosporins/β-lactamase inhibitors** |               |                  |              |
| Cefazidime (CAZ)                     | 14 (9.3%)     | 14 (9.3%)        | 122 (81.3%)  |
| Cephradine (CE)                      | 2 (1.3%)      | 1 (0.7%)         | 147 (98%)    |
| Cefoperazone (CFP)                   | 22 (14.7%)    | 5 (3.3%)         | 123 (82%)    |
| Cefoperazone-sulbactam (SCF)         | 38 (25.3%)    | 10 (6.7%)        | 102 (68%)    |
| Cefotaxime (CTX)                     | 14 (9.3%)     | 5 (3.3%)         | 131 (87.3%)  |
| Ceftriaxone (CRO)                    | 12 (8%)       | 6 (4%)           | 132 (88%)    |
| Cefuroxime (CXM)                     | 2 (1.3%)      | 2 (1.3%)         | 146 (97.3%)  |
| **Carbapenems**                      |               |                  |              |
| Doripenem (DOR)                      | 17 (11.3%)    | -                | 133 (88.7%)  |
| Ertapenem (ETP)                      | 5 (3.3%)      | -                | 145 (96.7%)  |
| Imipenem (IMI)                       | 38 (25.3%)    | 9 (6%)           | 103 (68.7%)  |
| Meropenem (MEM)                      | 43 (28.7%)    | 2 (1.3%)         | 105 (70%)    |
| **Aminoglycosides**                  |               |                  |              |
| Amikacin (AK)                        | 39 (26%)      | 7 (4.7%)         | 104 (69.3%)  |
| Gentamicin (GEN)                     | 8 (5.3%)      | -                | 142 (94.7%)  |
| Tobramycin (TOB)                     | 7 (4.7%)      | -                | 143 (95.3%)  |
| **Quinolones/fluoroquinolones**      |               |                  |              |
| Nalidixic acid (NAL)                 | 6 (4%)        | -                | 144 (96%)    |
| Ciprofloxacin (CIP)                  | 11 (7.3%)     | -                | 139 (92.7%)  |
| Levofloxacin (LEV)                   | 18 (12%)      | 3 (2%)           | 129 (86%)    |
| Ofloxacin (OFL)                      | 5 (3.3%)      | 3 (2%)           | 142 (94.7%)  |
| **Others**                           |               |                  |              |
| Aztreonam (ATM)                      | 13 (8.7%)     | 36 (24%)         | 101 (67.3%)  |
| Tigecycline (TGC)                    | 17 (11.3%)    | 21 (14%)         | 112 (74.7%)  |
| Tetracycline (TET)                   | 4 (2.7%)      | -                | 146 (97.3%)  |
| Polymyxin E (PE)                     | 83 (55.3%)    | -                | 67 (44.7%)   |
Table 2. ESBLs genotypes among PCR positive clinical isolates.

| PCR positive isolates | Number (%) | blaOXA | blaSHV | blaTEM | blaOXA+blaSHV | blaSHV+blaTEM | blaOXA+blaTEM | blaOXA+blaSHV+blaTEM |
|-----------------------|------------|--------|--------|--------|--------------|---------------|---------------|----------------------|
| Pseudomonas spp.,     | 29 (42%)   | 14 (48.2%) | 2 (6.8%) | 18 (62%) | -            | 2 (6.8%)      | 3 (10.3%)     | -                    |
| Klebsiella spp.,      | 23 (33.3%) | 6 (26%) | 9 (39.1%) | 21 (91.3%) | 3 (13%) | 8 (34.7%) | 2 (8.6%) | 3 (13%) |
| Acinetobacter spp.,   | 13 (18.8%) | 4 (30.7%) | 1 (7.6%) | 12 (92.3%) | - | 1 (7.6%) | 5 (38.4%) | - |
| Proteus spp.,         | 4 (5.7%)   | 2 (50%) | 1 (25%) | 4 (100%) | 1 (25%) | 1 (25%) | - | 1 (25%) |
| Total                 | 69 (100%)  | 26 (37.6%) | 13 (18.8%) | 55 (79.7%) | 4 (5.7%) | 12 (17.3%) | 10 (14.4%) | 4 (5.7%) |

Detection of blaOXA-, blaSHV-, and blaTEM-type ESBL genes

PCR screening of all the cephalosporin-resistant strains was performed to detect ESBL-encoding genes. There were 69 (46%) isolates that tested positive for ESBL genes. ESBL genes were identified in 29 (39.1%) Pseudomonas spp., 23 (67.6%) Klebsiella spp., 13 (43.3%) Acinetobacter spp., and 4 (33.3%) Proteus spp. strains. Multiplex PCR confirmed the coexistence of blaTEM and blaSHV genes in 12 (17.3%) patients. The coexistence of blaTEM and blaOXA genes was found in 10 (14.4%) patients. blaOXA, blaSHV and blaTEM coexistence was observed in only 4 (5.7%) patients. blaTEM genes were detected in 55 (79.7%) isolates, with the highest frequency of 21 (91.3%) in Klebsiella spp. isolated strains (Table 2).

Validity analysis

blaTEM genes were reported in 11 (20%) of the DDST-positive isolates, with 62% diagnostic accuracy (95% CI = 54.02, 69.3) and 86.3% specificity (77.9, 91.8), as shown in Table 3. These blaTEM genes were also confirmed in 10 (18.1%) CDT-positive isolates, with 62.6% diagnostic accuracy (95% CI = 54.7, 70) and 88.4% specificity (95% CI = 80.4, 93.4), as shown in Table 4.

Association of ESBL genes with antimicrobial resistance

All of the blaTEM-positive isolates were resistant to cefuroxime and 54 (98.1%) were resistant to cephradine, piperacillin, gentamicin, and tetracycline. Thirty-five (64.6%) blaTEM-positive isolates were resistant to meropenem and 27 (49%) were resistant to polymyxin E. All 26 of the blaOXA-positive isolates (100%) were resistant to amoxicillin-clavulanate and 25 (96%) were resistant to cephradine, cefuroxime, doripenem, ertapenem, ciprofloxacin and tetracycline. All 13 of the blaSHV-positive isolates (100%) were resistant to piperacillin and cefuroxime (Table 5).

Discussion

Multidrug resistance in Gram-negative isolates is found to be associated with the acquisition of β-lactamase gene variants [22]. B-lactamases are either encoded in the plasmids or chromosomal DNA [23]. ESBLs are known for hydrolyzing the penicillin, third and fourth generation cephalosporins and monobactams [10].
Table 4. Validity analysis of ESBLs detection by CDT as compared to multiplex PCR.

| Multiplex PCR | CDT Positive n (%) | CDT Not-determined n (%) | CDT Sensitivity (%) | CDT Specificity (%) | CDT Diagnostic accuracy (%) | Positive CDT predictive value (%) | Negative CDT predictive value (%) | Positive CDT likelihood ratio, 95% CI | Negative CDT likelihood ratio, 95% CI | χ² | p-value |
|---------------|---------------------|--------------------------|---------------------|---------------------|-----------------------------|-----------------------------------|-----------------------------------|--------------------------------------|--------------------------------------|-----|---------|
| *bla*<sub>ESBL</sub> positive | 5 (19.2%) | 21 (80.7%) | 19.2% | 87.1% | 75.3% | 23.8% | 83.7% | 1.49 | 0.9274 | 0.715 | 0.368 |
| *bla*<sub>ESBL</sub> negative | 16 (12.9%) | 108 (87.1%) | 1 (3.8%) | 1 (100%) | 12 (92.3%) | 5 (9%) | 1 (1.8%) | 54 (98.1%) |
| *bla*<sub>TEM</sub> positive | 2 (15.4%) | 11 (84.6%) | 15.4% | 86.1% | 80% | 9.5% | 91.5% | 1.09 | 0.9824 | 0.023 | 1.000 |
| *bla*<sub>TEM</sub> negative | 19 (13.8%) | 118 (86.1%) | 1 (7.6%) | 1 (7.6%) | 12 (92.3%) | 1 (1.8%) | 54 (98.1%) |
| *bla*<sub>SHV</sub> positive | 10 (18.2%) | 45 (81.8%) | 18.2% | 88.4% | 62.7% | 47.6% | 65.1% | 1.57 | 0.9253 |
| *bla*<sub>SHV</sub> negative | 5 (19.2%) | 21 (80.7%) | 19.2% | 87.1% | 75.3% | 23.8% | 83.7% | (0.5439 - 0.8832) | (0.9695 - 1.261) | 0.004565 | 1.067 |

Table 5. Association of ESBLs genes with the antimicrobial susceptibility patterns of clinical isolates.

| Antibiotics | *bla*<sub>CTX</sub> 26/150 (17.3%) | *bla*<sub>SHV</sub> 13/150 (8.6%) | *bla*<sub>TEM</sub> 55/150 (36.6%) |
|-------------|----------------------------------|----------------------------------|----------------------------------|
| PIP | 3 (11.5%) | - | 13 (100%) |
| AMC | - | 26 (100%) | 4 (7.2%) |
| SAM | 2 (7.6%) | 24 (92.3%) | 9 (69.2%) |
| TZP | 5 (19.2%) | 3 (11.5%) | 3 (23%) |
| CAZ | 1 (3.8%) | 4 (15.3%) | 11 (20%) |
| CE | - | 1 (3.8%) | 8 (61.5%) |
| CFP | 3 (11.5%) | 23 (88.4%) | 12 (92.3%) |
| SCF | 6 (23%) | 4 (15.3%) | 15 (27.2%) |
| CTX | 3 (11.5%) | 22 (84.6%) | 12 (92.3%) |
| CRO | 1 (3.8%) | 2 (7.6%) | 10 (76.9%) |
| CMX | - | 25 (96.1%) | 12 (92.3%) |
| DOR | 1 (3.8%) | 25 (96.1%) | 1 (1.8%) |
| ETP | 1 (3.8%) | 25 (96.1%) | 12 (92.3%) |
| IMI | 7 (26.9%) | 1 (3.8%) | 17 (30.9%) |
| MEM | 9 (34.6%) | 17 (65.3%) | 20 (36.3%) |
| AK | 6 (23%) | 2 (7.6%) | 17 (30.9%) |
| GEN | 2 (7.6%) | 24 (92.3%) | 12 (92.3%) |
| TOB | 2 (7.6%) | 24 (92.3%) | 12 (92.3%) |
| NAL | 2 (7.6%) | 24 (92.3%) | 12 (92.3%) |
| CIP | 1 (3.8%) | 25 (96.1%) | 12 (92.3%) |
| LEV | 3 (11.5%) | 23 (88.4%) | 12 (92.3%) |
| OFL | 1 (3.8%) | 24 (92.3%) | 12 (92.3%) |
| ATM | 1 (3.8%) | 11 (42.2%) | 9 (69.2%) |
| TGC | 4 (15.3%) | 3 (11.5%) | 7 (53.8%) |
| TET | 1 (3.8%) | 25 (96.1%) | 13 (100%) |
| PE | 13 (50%) | 13 (50%) | 7 (53.8%) |

S: Sensitive; I: Intermediate; R: Resistant; PIP: Pipacillin; AMC: Amoxicillin-clavulanate; SAM: Ampicillin-sulbactam; TZP: Pipacillin-tazobactam; CAZ: Cefazidine; CE: Cephradine; CFP: Cefoperazone; SCF: Cefoperazone-sulbactam; CTX: Cefotaxime; CRO: Ceftriaxone; CMX: Cefuroxime; DOR: Doripenem; ETP: Ertapenem; IM: Imipenem; MEM: Meropenem; AK: Amikacin; GEN: Gentamicin; TOB: Tobramycin; NAL: Naldixic acid; CIP: Ciprofloxacin; LEV: Levofloxacin; OFL: Ofloxac, ATM: Aztreonam; TGC: Tigecycline; TET: Tetracycline, and PE: Polymyxin E.
Ambler classification involves four classes of β-lactamases including class A, B, C, and D [24]. Class A ESBLs such as sulphydryl variant (SHV) and Temorina Escherichia coli mutant (TEM) are inactivated by β-lactamases including clavulanic acid, sulbactam, tazobactam [25].

Particular types of ESBLs are also capable of inactivating the aminoglycosides and sulphonamides [26]. Class D oxacillinases such as OXA type ESBLs and carbapenemases including \( \text{bla}_{\text{OXA-10}} \) and \( \text{bla}_{\text{OXA-48}} \) respectively, are capable of degrading the cephalosporins and carbapenems respectively [27]. Cephamycins and carbapenems are resistant to degradation by ESBLs [24].

This study includes assessment of cephalosporins and carbapenems resistance in burn 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 no significant difference observed between the community-acquired (50.7%) and nosocomial infections (49.3%). Community acquired infections were less prevalent about a decade ago where only 16.9% 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 easy access to the commercially available antibiotics and inappropriate prescriptions by physicians may be the contributing factors in the emergence of MDR strains [12,24]. Individuals with the young age work in different factories and industries. Most of the burn victims belonged to the young age of 20-30 years as previously the similar age groups of burn patients have been observed in Iran [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 the multiple bacterial strains have been isolated from the burn patients with *Pseudomonas* spp., and *Acinetobacter* spp. co-infection [32].

There were 83.3% (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 69% resistance against imipenem. ESBLs positive *P. aeruginosa* isolates have been observed in burn patients in Pakistan with 61% resistance against imipenem [13]. *E. coli* strains with 47% and *K. pneumoniae* with 44% resistance against meropenem have been detected in burn patients in Nigeria [18]. Early investigations on burn patients differ where more than 80% isolates were resistant to imipenem and meropenem. Burn patients in Sofia, Bulgaria have been reported with 82-85% resistance against imipenem and meropenem in *A. baumannii* isolates [32,33]. The cephradine resistance was observed in 98% of the isolates while the remaining 2% were found to be resistant against cefoperazone and cefotaxime. The phenotypic detection of all the isolates was not helpful as DDST and CDT negative but cephalosporins resistant isolates need to be tested by molecular techniques. Molecular detection by multiplex PCR is the gold standard and more sensitive as 46% of cephalosporins resistant isolates were positive for ESBLs genes as compared to 24% ESBLs confirmed by phenotypic testing. 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 advance molecular techniques for the rapid, specific and accurate diagnosis of ESBLs producers [34].

\( \text{bla}_{\text{TEM}} \) was predominant in cephalosporins resistant isolates followed by \( \text{bla}_{\text{OXA}} \) and \( \text{bla}_{\text{SHV}} \). These findings are in agreement with Bajpai et al., form New Delhi, India where \( \text{bla}_{\text{TEM}} \) was detected in 48.7% isolates followed by \( \text{bla}_{\text{SHV}} \) [35]. Shakibaie et al., reported 6.6% \( \text{bla}_{\text{SHV}} \) and of 2.5% \( \text{bla}_{\text{TEM}} \) from burn patients in Iran [30]. The differences in the occurrence of ESBLs genes are based on their geographical distribution as \( \text{bla}_{\text{TEM}} \) is predominant in China and \( \text{bla}_{\text{SHV}} \) is the leading ESBL in North America [10]. Co-existence of ESBLs genes was confirmed in our findings where \( \text{bla}_{\text{SHV}} + \text{bla}_{\text{TEM}} \) were detected in 17.39% and \( \text{bla}_{\text{OXA}} + \text{bla}_{\text{TEM}} \) in 14.5 PCR positive isolates. These findings differ from Parajuli et al., where \( \text{bla}_{\text{SHV}} \) and \( \text{bla}_{\text{TEM}} \) co-existed in 10% isolates [36]. \( \text{bla}_{\text{OXA}}, \text{bla}_{\text{SHV}}, \) and \( \text{bla}_{\text{TEM}} \) co-existence was detected in 5.8% isolates which indicates the parallel emergence of different ESBLs in the same isolates from burn patients and their surroundings.

The clinical presentation of patients suffering from post-burn infections is very difficult to interpret which marks inappropriate prescription of antibiotics. Multi-drug resistance in burn isolates develops due to slow
wounds healing associated hospitalizations poor diagnosis and treatment. There is an urgent need to assess the incidence of post-burn infections by multidrug resistant bacterial pathogens. 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 promising that ensures cost-effectiveness and speedy output as compared to traditional culturing and phenotypic tests. Multiplex PCR can be used 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. The emergence of Pseudomonas spp. as a predominant pathogen, followed by Enterobacteriaceae, is becoming problematic in the treatment of burn patients. Phenotypic testing of ESBLs is less effective due to the lower sensitivity of DDST and CDT. Molecular detection of ESBL-encoding genes by PCR is the more accurate, reliable, and specific diagnostic method. blaTEM, followed by blaOXA, ESBLs are frequently associated with the inefficacy of third-generation cephalosporins and carbapenems. blaSHV ESBLs were less in number, but all of these isolates were resistant to piperacillin. It is necessary to overcome the emergence of multidrug-resistant bacterial pathogens by understanding their antimicrobial resistance mechanisms other than the enzymatic degradation of antibiotics. The spread of multidrug-resistant strains in clinical burn settings is alarming; therefore, treatment strategy and infection-control management should be improved immediately.

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Authors’ contributions
Study concept and study design: SR; data collection: SB and MH; NuA, SA (helped in the management of data and strains from Allama Iqbal Medical College); manuscript review and editing: NuA, SA and NM; Major experimental work: MH and SR. All authors approved the final version of the manuscript.

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

Declaration
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**Corresponding author**

Dr Saba Riaz  
Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan, 21, Lahore, 5400, Punjab, Pakistan.  
Tel.:92-3364208516  
Fax: 92-42-35952855  
Email: saba.mmg@pu.edu.pk

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