Community Acquired Multi-drug Resistant Clinical Strains from Tracheal Aspirates of Patients in Hospital Settings in Dhaka, Bangladesh

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Abstract

Background: Antimicrobial resistance is a multi-sectoral problem which poses a major threat in the treatment of infectious diseases especially in developing countries like Bangladesh. Multidrug-resistant (MDR) bacteria along with extremely drug resistant (XDR) bacteria have emerged as major clinical and therapeutic dilemma in the treatment of tracheal infections in hospitals here. Thus the aim of this study was to document the incidence of MDR and XDR producing β-lactamases in clinical isolates from tracheal aspirates of patients in Dhaka, Bangladesh.

Methods: Two hundred clinical isolates from tracheal aspirates were identified and their antibiotic susceptibility profiles were evaluated by using the VITEK 2 system following the Clinical and Laboratory Standards Institute guidelines. Patient information on diagnosis, sex, age was obtained from hospital data.

Results: Of 200 clinical, non-duplicate bacterial isolates obtained, Pseudomonas aeruginosa was the most frequent pathogens (N=61/200, 30.5%) followed by Acinetobacter baumannii (N=58/200, 29%), Klebsiella pneumoniae (N=45/200, 22.5%), Streptococcus pneumoniae (N = 15/200, 7.5%), Escherichia coli (N=10/200, 5%), Staphylococcus aureus (N=4/200, 2%), Proteus spp (N=3/200, 1.5%), Enterobacter spp (N=2/100, 1%), Citrobacter spp (1/200, 0.5%), Providencia spp (N=1/200, 0.5%). Of 20 different antibiotics tested, highest number of isolates (N=172/200, 86%) showed resistance to third generation cephalosporin cefixime, however least number of isolates showed resistance to polymixin antibiotics-colistin (N=25/200, 12.5%) and polymixinB (N=12/200, 6%). The patients’ ages ranged between 1 month to 95 years with the gender distribution of 133 (66.5%) males and 67 (33.5%) females. The prevalence of infections was highest among the
patients of age-group (old adults) ≥60 years (N=123/200, 61.5%). Of 200 clinical isolates, 43 (21.5%) were XDR and 125 (62.5%) were MDR bacteria. Of 200 clinical isolates, the synthesis of extended spectrum β-lactamases (ESBL) and carbepenemase were detected in 59 (29.5%) and 98 (49%) strains respectively.

Conclusion: Tracheal infections caused by MDR and XDR pathogens among patients are high at hospital settings in Bangladesh. Therefore, there is an urgent need for constant surveillance and interventions in Bangladesh in order to prevent further spreading of those resistant organisms.

Introduction

Respiratory infections are the leading cause of global morbidity and mortality from infectious diseases worldwide [1]. Community acquired pneumonia (CAP), nosocomial pneumonia and acute and chronic bronchial infections in patients with chronic obstructive pulmonary disease (COPD) and bronchiectasis are known as the most common respiratory diseases those are responsible for elevated morbidity and mortality rate [2]. Lower respiratory tract infections like tracheal infections are caused by both of Gram-positive and Gram-negative bacteria. The emergence of multidrug-resistant (MDR) bacteria poses a major threat in hospital settings [3]. The most frequent multidrug-resistant bacteria associated with tracheal infections are Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Acinetobacter baumannii, and other Enterobacteriaceae [4].

Antibiotic resistance is an increasingly serious threat to global public health that threatens our ability to treat common infectious diseases, resulting in prolonged illness, disability and death (5). In recent years, several studies have reported an increased number of bacteria causing both hospital-acquired and community-
acquired infections [3, 6]. Enterobacteriaceae including K. pneumoniae, E. coli as well as Enterobacter spp. along with other bacteria such as P. aeruginosa and A. baumannii have been identified as major cause of multi-drug resistant (MDR) and extremely drug resistant (XDR) bacterial infections in respiratory tract [6-9]. However, Gram-positive organisms such as Staphylococcus aureus which is a common causative agent of severe infections in health facilities and in the community become resistant to first-line drugs [5]. Patients infected with methicillin-resistant Staphylococcus aureus (MRSA) are estimated to be 64% more likely to die than people with a non-resistant form of the infection whereas MRSA are also reported to cause tracheal infections [5, 10]. For the treatment of life-threatening infections caused by Enterobacteriaceae which are resistant to carbapenems, colistin is used as the last resort of treatment [11]. However, resistance to colistin has been detected recently in several countries, making infections untreatable those are caused by such bacteria [5, 11]. Resistance to broad spectrum β-lactams mediated by extended spectrum β-lactamases (ESBL) is a global threat [12]. The emergence of ESBL along with carbapenemases is caused by using β-lactam antibiotics extensively over the last several decades in the clinical practice [13]. New variants of β-lactamases have emerged due to the selective pressure imposed by the use and overuse of new antibiotics in the treatment of patients [14]. Most ESBL producing organisms are also resistant to aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol and sulfonamides as they have large plasmids where ESBL genes along with other antimicrobial resistant genes are present [13]. This study aimed to assess multidrug-resistance among Gram-negative and Gram-positive bacteria those are responsible to cause lower respiratory tract infections in
Dhaka, Bangladesh to guide treatment protocols along with to determine the existence of ESBL, carbapenemase production in multi-drug and extensively-drug resistant bacterial strains isolated from tracheal aspirates. The data further provides a baseline for future comparative studies.

Materials and Methods

Study:

The study was conducted between January 2018 and June 2019, in Dhaka Central International Medical College and Hospital in Dhaka, Bangladesh from where tracheal aspirates specimens (N=200) were aseptically collected from patients (N=200; Male=133, Female=67) then subsequently transported to microbiology laboratory of Primasia University for bacterial isolation and identification, phenotypic determination of antibiotic susceptibility, identification of multidrug resistant (MDR), extremely drug resistant (XDR), pan-drug resistant (PDR) organisms along with detection of ESBL and carbapenemase production. Information on diagnosis, sex, age was obtained from patients’ records.

Bacterial Strains

Of 200 tracheal aspirates, 149 samples showed bacterial growth whereas 51 were sterile. Of 149 samples, total of 200 clinical, non-duplicate bacteria were isolated those were maintained on nutrient agar slants, frozen in lyophilizing medium at −70 °C. The identification of bacterial isolates and the evaluation of their antibiotic susceptibility profiles were performed using the VITEK 2 system (bioMérieux, Inc., Hazelwood, MO, United States) following the Clinical and Laboratory Standards Institute guidelines [15].

Antimicrobial drug susceptibility testing
Antimicrobial drug susceptibility testing was conducted by Kirby–Bauer method in accordance with the Clinical and Laboratory Standards Institute [15] against penicillins with β-lactamase inhibitors [amoxicillin-clavulanic acid (10 µg), piperacillin-tazobactam (100/10 µg)], cephalosporin [cefuroxime (10 µg), cefixime (5 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefepime (30 µg)], monobactam [aztreonam (30 µg)], carbapenems [imipenem (10 µg), meropenem (10 µg)], aminoglycosides [gentamicin (10 µg), amikacin (30 µg), netilmicin (10 µg)], fluoroquinolones [ciprofloxacin (5 µg), levofloxacin (5 µg)], folate pathway inhibitor [co trimoxazole (25 µg)], polymyxin [colistin (10 µg), polymyxin B (300U)], glycylcyclines [tigecyclin, (15 µg)]. Methicillin (5 µg) is used only against S. aureus. Susceptibility to tigecyclin was interpreted using breakpoints proposed by the European Committee on Antimicrobial Susceptibilities Testing (EUCAST) [16]. The combination disk test using cefotaxime and ceftazidime, alone and in combination with clavulanic acid was performed in accordance with Clinical and Laboratory Standards Institute guidelines for detection of ESBL (1). Determination of the production of carbapenemase was carried out by modified Hodge test and imipenem-EDTA disk synergy test as described [15, 17]. MDR, XDR and PDR isolates were identified according to the guidelines recommended by joint initiative of the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) [18].

Results

Of 200 clinical, non-duplicate bacterial isolates obtained, P. aeruginosa was the most frequent pathogens (N=61/200, 30.5%) followed by A. baumannii (N=58/200, 29%), K. pneumoniae (N=45/200, 22.5%), S. pneumoniae (N=15/200, 7.5%), E. coli
(N=10/200, 5%), S. aureus (N=4/200, 2%), Proteus spp(N=3/200, 1.5%), Enterobacter spp (N=2/100, 1%), Citrobacter spp (1/200, 0.5%), Providencia spp (N=1/200, 0.5%) (Table 1) (Figure 1).

Of 20 different antibiotics tested, highest number of isolates (N=172/200, 86%) showed resistance to third generation cephalosporin cefixime, however least number of isolates showed resistance to polymixin antibiotics- colistin (N=25/200, 12.5%) and polymixin B (N=12/200, 6%) (Table 2). 83% A. baumannii (N=48/58) were found to be resistant to amoxicillin-clavulanic acid and cefixime whereas only 12% (N=7/58) showed resistance to polymixin B (Table 3). All strains of E. coli were found to be sensitive to polymixin B though 90% (N=9/10) of those strains were resistant to amoxicillin-clavulanic acid. 87% strains of K. pneumonia (N=39/45) showed resistancne to cefixime and cefuroxime, however 2% (N=1/45) were resistant to polymixin B. 87% strains of P. aeruginosa (N=53/61) were resistant to cefotaxime and cefixime. 93% strains of S. pneumoniae (N=14/15) were resistant to cefixime. All strains of S. aureus (N=4/4, 100%) were found to resistant to methicillin though those were sensitive to co-trimoxazole, colistin, polymixin B, tigecyclin, gentamicin, netilmicin (Table 3).

**Demographic characteristics of patients with bacterial infections**

The patients’ ages ranged between 1 month to 95 years with the gender distribution of 133 (66.5%) males and 67 (33.5%) females (Figure 2). The prevalence of infections was highest among the patients of age-group (old adults) ≥60 years (N=123/200, 61.5%) followed by middle aged adults (50-59 years) 12% (N=24/200), young adults (30-39 years) 6% (N=12/200), baby (0-2 years) 5.5% (N=11/200), in 3-12 years 5% (N=10/200), in 20-29 years 3.5% (N=7/200). The least prevalence rate (N=2/200, 1%) was found in young adults of age group 13-19 years (Table 5).
**Figure 3.** Tracheal infection was found to be more prevalent in males rather than in females (Figure 2). The highest prevalence of infections caused by *A. baumannii* was in males (42/58, 72.4%) than in females (N=16/58, 27.58%). The prevalence of both *P. aeruginosa* and *K. pneumoniae* were higher in males (N=38/61, 62.3%; N=30/45, 66.67%) than in females (N=23/61, 37.7%; N=15/45, 33.33%) (Table 6) (Figure 2).

**XDR and MDR Strains:**

Of 200 bacterial isolates obtained from tracheal aspirates, 68% (N=136/200) isolates were MDR whereas 22% (N=43/200) were XDR (Figure 4). Of 61 *P. aeruginosa* strains tested, 14 (23%) were XDR and 43 (70.4%) were MDR organisms whereas of 58 strains of *A. baumannii*, 33 (57%) were MDR and 17 (29%) were XDR. Of 45 *K. pneumoniae*, 32 (71.1%) were MDR and 9 (20%) were XDR (Figure 5). For *S. pneumoniae*, all strains (N=12/15, 80%) were MDR among which 1 strain (7%) was XDR though 1 strain was found to be sensitive to all drugs. Of 10 strains *E. coli*, 5 strains (50%) were MDR and 2 were XDR (20%). For *S. aureus, Proteus spp, Enterobacter spp, Citrobacter spp* and *Providencia spp*, all strains were found to be MDR (Table 4).

**Extended Spectrum β-Lactamase (ESBL) and Carbapenemase Producing Strains:**

Of 200 clinical isolates, the synthesis of ESBL and carbapenemase were detected in 59 (29.5%) and 98 (49%) strains respectively. Of 58 strains of *A. baumannii*, 28 (48%) and 18 (31%) strains produced carbapenemase and ESBL respectively (Table 7) (Figure 6). Of 45 strains of *K. pneumonia*, carbapenemase and ESBL were detected in 22 (49%) and 16 (36%) strains respectively. Of 10 *E. coli* strains, 5 (50%) and 3 (30%) strains produced carbapenemase and ESBL respectively.
Carbapenemase production was found in \textit{S. pneumoniae} (N=4/15, 27%), \textit{Proteus spp.} (N=2/3, 67%), \textit{Citrobacter spp} (N=1/1, 100%) and in \textit{Providencia spp} (N=1/1, 100%) though no ESBL production was found (Table 7). Most of the antibiotics tested were non-effective against ESBL and carbapenemase producer whereas polymixin B, colistin, tigecyclin were found to be effective regimens against ESBL and carbapenemase producers.

\textbf{Discussion}

Antimicrobial resistance (AMR) is a major problem to global public health that requires action across all government sectors and society [5]. Infections caused by resistant bacteria responsible for longer duration of illness, additional tests and use of more expensive drugs rather than those infections which are caused by nonresistant bacterial species [5]. Epidemiological surveillance of resistance to antibiotics is essential in developing countries like Bangladesh where infections caused by multi-drug resistant bacteria which have resulted in increased morbidity and mortality [19–20]. Antibiotic resistance is an increasingly serious threat in Bangladesh which is most likely a result of unrestricted use of antimicrobial drugs [21–22].

Our study observed the prevalence of tracheal infections was highest among the patients of old adults whose age was $\geq$60 years; however least susceptibility to these infections was noticed in young adults of age group 13–19 years. The annual incidence of pneumonia in the elderly people is four-times higher than that of younger populations reported elsewhere [23]. Tracheal infection was found to be more prevalent in males rather than in females.

Tracheal infection in patients was caused mainly by \textit{P. aeruginosa} (30.5%) followed
by A. baumannii (29%). Since P. aeruginosa is an opportunist, it can colonize the respiratory tracts after endotracheal intubation or in critically ill and immunocompromised patients [24], especially in cystic fibrosis patients [25] where it can be aspirated into the lungs [26]. Tracheal intubation and use of carbapenems are considered as risk factors for patients with P. aeruginosa infection [24]. A report published in 2016 showed that 33.9% of P. aeruginosa were resistant to at least one of the antimicrobial groups under surveillance in Europe [27]. Our study found 23% strains of P. aeruginosa were XDR and 70.4% were MDR organisms whereas 87% strains of P. aeruginosa were resistant to cefotaxime and cefixime.

A. baumannii is an important nosocomial pathogen in healthcare facilities and has become one of the most significant microorganisms causing infections in hospitalized patients in last few decades [28]. A study conducted at the Dhaka Medical College Hospital (DMCH) showed 96% strains of A. baumannii isolated from endotracheal aspirates collected from patients, were multidrug resistant [29]. Another study carried in Square Hospitals Ltd. showed 90% of the A. baumannii strains isolated from the patients with lower respiratory tract infections, were multidrug resistant [30]. In our study, 57% A. baumannii were MDR and 29% were XDR indicating an alarming situation. Moreover, 83% A. baumannii were found to be resistant to amoxicillin-clavulanic acid and cefixime whereas only 12% showed resistance to polymixin B.

The highest incidence rate of respiratory tract infection was caused by A. baumannii (25%) followed by Pseudomonas spp. (15%) and Klebsiella spp. (10%) [31]. Our findings correlate with these reports though P. aeruginosa was to be found as a predominate organisms causing respiratory tract infections. In our study, it was observed that among 45 strains of K. pneumoniae, 71% were MDR and 20% were
XDR. *K. pneumoniae* was the most common causative agent of nosocomial pneumonia where the presence of MDR *K. pneumoniae* strains was prevalent [32]. However, other studies showed the most prevalent organism causing tracheal infections was *Enterobacter* spp. followed by *P. aeruginosa* [33–34]. Though only 1% strains causing tracheal infection were *Enterobacter* spp in our study, those were multidrug resistant. Moreover, it was observed methicillin resistant *S. aureus* (MRSA) was found to be responsible for tracheal infections. A report stated elsewhere that MRSA is a cause of lung infection including airway infection, community-acquired pneumonia and hospital-acquired pneumonia [35]. Among the Gram-negative bacteria causing chronic respiratory disease, *E. coli* is considered one of the major respiratory threats [36]. Our study showed 20% *E. coli* strains were XDR and 50% were MDR. *S. pneumoniae* is an important causative agent of chronic respiratory disease including tracheal infections that result in higher rate of morbidity and mortality due to MDR *S. pneumoniae* [37]. It was observed 93% strains of *S. pneumoniae* causing tracheal infection were resistant to cefixime whereas 80% strains were MDR.

The present study observed highest number of strains of both Gram-positive and Gram-negative bacteria showed resistance to third generation cephalosporins, however the most effective antibiotics were polymixin antibiotics especially colistin and polymixin B along with tigecyclin. These findings correlate with other reports where colistin was reported as an effective drug in the treatment of infections caused by MDR bacteria [38–39].

In the recent years, antimicrobial resistance mediated by ESBL- and carbapenemase has been found to be ubiquitous [40] and the current dissemination of these enzymes makes it mandatory to understand this phenomenon especially because of
the higher mortality, morbidity, and increased health treatment costs associated with resistance to β-lactams [41]. The increasing rate of dissemination of carbapenemase in Bangladesh has been documented with the isolation of clinical A. baumannii, P. aeruginosa and K. pneumonia [42]. The present study showed the synthesis of ESBL and carbapenemase were detected in 29.5% and 49% strains respectively where it was noticed most of the antibiotics tested were non-effective against ESBL and carbapenemase producer, however, polymixin B, colistin, tigecyclin were found as effective antibiotics against ESBL and carbapenemase producers.

Conclusion

The study demonstrated high prevalence of β-lactamase producing multidrug resistant bacteria implicated in the tracheal infections diagnosed among patients. Infections were common among the elderly people and predominantly caused by P. aeruginosa followed by A. baumannii, K. pneumonia, Streptococcus spp during the period of our study. Appropriate and justified use of antimicrobial agents should be ensured in controlling the growing danger of antimicrobial drug resistance. Therefore, there is an urgent need for constant surveillance and interventions in Bangladesh in order to prevent further spreading of those resistant organisms. Further studies at molecular level will be required to determine the mechanism(s) of resistance by genotypic methods.

Declarations

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**Competing interests:**

The authors declare that they have no competing interests.

**Availability of data and materials:**

Additional information of the study can be made available from the corresponding author on request where necessary.

**Authors’ contributions:**

The study was co-conceptualized and jointly designed by FTJ, JA, TF and MMHS. JA collected the data and undertook laboratory analysis with the help from SS, TF and MJF. FTJ and MMHS analyzed and interpreted the data with assistance from AKD, SA, MZR, ANC and MM. All the authors contributed in preparation and submission of manuscript. All authors read and approved the final manuscript.

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**Ethical approval and consent to participate:**

Ethical clearance was approved by Ethical Committee, Bangladesh Council of Scientific and Industrial Research (BCSIR), Rajshahi. Verbal consent was taken from
all participants in this study and from parents or guardians for minors’ patients
after explanation of the procedure and the purpose of the study.

Consent for publication:
Not applicable

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Tables

Table 1: Bacterial isolates from tracheal aspirates specimen

| Bacterial Isolates | Total Number of Isolates (N) | Number of Isolated Organisms N (%) |
|--------------------|-------------------------------|-----------------------------------|
| *P. aeruginosa*     | 200                           | 61 (30.5)                         |
| *A. baumannii*      | 58 (29)                       |                                   |
| *K. pneumoniae*     | 45 (22.5)                     |                                   |
| *S. pneumoniae*     | 15 (7.5)                      |                                   |
| *E. coli*           | 10 (5)                        |                                   |
| *S. aureus*         | 4 (2)                         |                                   |
| *Proteus spp*       | 3 (1.5)                       |                                   |
| *Enterobacter spp*  | 2 (1)                         |                                   |
| *Citrobacter spp*   | 1 (0.5)                       |                                   |
| *Providencia spp*   | 1 (0.5)                       |                                   |

Table 2: Resistance rate of isolates to different antibiotics

| Antibiotics | Total Number of Isolates (N) | Susceptible N (%) | Intermediate N (%) |
|-------------|-------------------------------|-------------------|-------------------|
| Penicillins | 200                           |                   |                   |
| Penicillin with β-lactamase Inhibitors | | | |
| Amox/Clav   | 38 (19)                       | 3 (1.5)           |                   |
| Piperacillin/Tazobactam | 102 (51) | 12 (6) | |
| Cephalosporins | | | |
| Second Generation | | | |
| Cefuroxime  | 32 (32)                       |                   | 6 (3)             |
| Antibiotic          | Sensitivity | Resistance |
|---------------------|-------------|------------|
| **Third Generation**|             |            |
| Cefixime            | 28 (14)     | 0 (0.0)    |
| Cefotaxime          | 36 (18)     | 5 (2.5)    |
| Ceftazidime         | 73 (36.5)   | 10 (5)     |
| Ceftriaxone         | 40 (20)     | 7 (3.5)    |
| **Fourth Generation**|             |            |
| Cefepime            | 83 (41.5)   | 8 (4)      |
| **Aminoglycosides**|             |            |
| Amikacin            | 80 (40)     | 5 (2.5)    |
| Gentamicin          | 91 (45.5)   | 0          |
| Netilmicin          | 92 (46)     | 1 (0.5)    |
| **Carbapenems**     |             |            |
| Imipenem            | 85 (42.5)   | 3 (1.5)    |
| Meropenem           | 92 (46)     | 1 (0.5)    |
| **Monobactams**     |             |            |
| Aztreonam           | 49 (24.5)   | 11 (5.5)   |
| **Fluoroquinolones**|             |            |
| Ciprofloxacin       | 68 (34)     | 12 (6)     |
| Levofloxacin        | 80 (40)     | 5 (2.5)    |
| **Folate Pathway Inhibitors** | | |
| Co-trimoxazole      | 77 (38.5)   | 4 (2)      |
| **Polymixins**      |             |            |
| Colistin            | 173 (86.5)  | 2 (1)      |
| Polymixin B         | 188 (94)    | 0 (0.0)    |
| **Glycylcyclines**  |             |            |
| Tigecyclin          | 136 (68)    | 24 (12)    |

Table 3: Resistance pattern of isolates to individual antibiotics
| Antibiotics             | No. of Resistant Isolates (%) | A. baumannii (N=58) | E. coli (N=10) | K. pn |
|------------------------|-------------------------------|---------------------|----------------|-------|
| Amox/Clav              | 159 (79.5)                    | 48 (83)             | 9 (90)         | 34    |
| Piperacillin/Tazobactam| 86 (43)                       | 28 (48)             | 4 (40)         | 19    |
| Cefuroxime             | 162 (81)                      | 45 (78)             | 7 (70)         | 39    |
| Cefixime               | 172 (86)                      | 48 (83)             | 8 (80)         | 39    |
| Cefotaxime             | 159 (79.5)                    | 44 (76)             | 6 (60)         | 37    |
| Ceftazidime            | 117 (58.5)                    | 31 (53)             | 4 (40)         | 29    |
| Ceftriaxone            | 153 (76.5)                    | 42 (72)             | 7 (70)         | 35    |
| Cefepime               | 109 (54.5)                    | 29 (50)             | 4 (40)         | 25    |
| Amikacin               | 115 (57.5)                    | 32 (55)             | 5 (50)         | 22    |
| Gentamicin             | 109 (54.5)                    | 32 (55)             | 7 (70)         | 28    |
| Netilmicin             | 101 (50.5)                    | 30 (52)             | 5 (50)         | 25    |
| Imipenem               | 112 (56)                      | 33 (57)             | 7 (70)         | 23    |
| Meropenem              | 107 (53.5)                    | 32 (55)             | 7 (70)         | 23    |
| Aztreonam              | 140 (70)                      | 39 (67)             | 7 (70)         | 35    |
| Ciprofloxacin          | 120 (60)                      | 33 (57)             | 6 (60)         | 28    |
| Levofoxacin            | 115 (57.5)                    | 33 (57)             | 6 (60)         | 26    |
| Co-trimoxazole         | 119 (59.5)                    | 38 (66)             | 4 (40)         | 29    |
| Colistin               | 25 (12.5)                     | 10 (17)             | 2 (20)         | 4     |
| Polymixin B            | 12 (6)                        | 7 (12)              | 0 (0)          | 1     |
| Tigecyclin             | 40 (20)                       | 12 (21)             | 2 (20)         | 7     |

Table 4: Prevalence of MDR and XDR isolates causing tracheal infections
| Bacterial Isolates (N) | Number of Isolated Organisms N | No. of MDR Organisms N (%) | No. of XDR Organisms N (%) |
|------------------------|--------------------------------|-----------------------------|-----------------------------|
| *P. aeruginosa*        | 61                             | 43 (70.49)                  | 14 (23)                     |
| *A. baumannii*         | 58                             | 33 (56.89)                  | 17 (29)                     |
| *K. pneumonialae*      | 45                             | 32 (71.1)                   | 9 (20)                      |
| *S. pneumonialae*      | 15                             | 12 (80)                     | 1 (7)                       |
| *E. coli*              | 10                             | 5 (50)                      | 2 (20)                      |
| *S. aureus*            | 4                              | 4 (100)                     | 0 (0)                       |
| *Proteus spp*          | 3                              | 3 (100)                     | 0 (0)                       |
| *Enterobacter spp*     | 2                              | 2 (100)                     | 0 (0)                       |
| *Citrobacter spp*      | 1                              | 1 (100)                     | 0 (0)                       |
| *Providencia spp*      | 1                              | 1 (100)                     | 0 (0)                       |
| Frequency              | 200                            | 136 (68)                    | 43 (22)                     |

Table 5: Distribution of different isolates among different age group of patients

| Age Group        | Age Intervals | No. of Patients (N=200) (%) | *A. baumannii* (N=58) (%) | *E. coli* (N=10) (%) | *K. pneumonialae* (N=45) (%) |
|------------------|---------------|-----------------------------|---------------------------|----------------------|-----------------------------|
| Baby             | 0-2           | 11 (5.5)                    | 1 (1.7)                   | 2 (20)               | 2 (4.5)                     |
|                  | 3-12          | 10 (5)                      | 10 (17.3)                 | 2 (20)               | 3 (6.7)                     |
|                  | 13-19         | 2 (1)                       |                           |                      |                             |
|                  | 20-29         | 7 (3.5)                     |                           |                      |                             |
|                  | 30-39         | 12 (6)                      |                           |                      |                             |
| Mid Aged Adults  | 40-49         | 11 (5.5)                    | 9 (15.5)                  | 1 (10)               | 6 (13.3)                    |
|                  | 50-59         | 24 (12)                     |                           |                      |                             |
| Old Adults       | 60-69         | 44 (22)                     | 38 (65.5)                 | 5 (50)               | 34 (75.5)                   |
|                  | 70-79         | 42 (21)                     |                           |                      |                             |
|                  | 80-89         | 25 (12.5)                   |                           |                      |                             |
|                  | 90-99         | 12 (6)                      |                           |                      |                             |
|                  | 100-Above     | 0 (0)                       |                           |                      |                             |
Table 6: Distribution of isolates according to the patients’ gender

| Bacterial Isolates (N) | Number of Isolated Organisms N | Male    | Female   |
|------------------------|---------------------------------|---------|----------|
| *P. aeruginosa*        | 61                              | 38 (62) | 23 (38)  |
| *A. baumannii*         | 58                              | 42 (72) | 16 (28)  |
| *K. pneumoniae*        | 45                              | 30 (67) | 15 (33)  |
| *S. pneumoniae*        | 15                              | 7 (47)  | 8 (53)   |
| *E. coli*              | 10                              | 9 (90)  | 1 (10)   |
| *S. aurues*            | 4                               | 3 (75)  | 1 (25)   |
| *Proteus spp*          | 3                               | 1 (33)  | 2 (67)   |
| *Enterobacter spp*     | 2                               | 1 (50)  | 1 (50)   |
| *Citrobacter spp*      | 1                               | 1 (100) | 0 (0)    |
| *Providencia spp*      | 1                               | 1 (100) | 0 (0)    |
| Frequency              | 200                             | 133 (66.5) | 67 (33.5) |

Table 7: Distribution of ESBL and carbapenemase production in different isolates
### Types of β-lactamase Production

| Bacterial Isolates (N) | Number of Isolated Organisms N | Carbapenemase | ESBL |
|------------------------|--------------------------------|---------------|------|
| *P. aeruginosa*        | 61                             | 35 (57)       | 22 (36) |
| *A. baumannii*         | 58                             | 28 (48)       | 18 (31) |
| *K. pneumoniae*        | 45                             | 22 (49)       | 16 (36) |
| *S. pneumoniae*        | 15                             | 4 (27)        | 0 (0)  |
| *E. coli*              | 10                             | 5 (50)        | 3 (30) |
| *S. aureus*            | 4                              | 0 (0)         | 0 (0)  |
| *Proteus spp*          | 3                              | 2 (67)        | 0 (0)  |
| *Enterobacter spp*     | 2                              | 0 (0)         | 0 (0)  |
| *Citrobacter spp*      | 1                              | 1 (100)       | 0 (0)  |
| *Providencia spp*      | 1                              | 1 (100)       | 1 (100) |
| Frequency              | 200                            | 98 (49)       | 59 (29.5) |

**Figures**
All species causing tracheal infections were showed in the pie chart. The left pie chart shows:

- 29% of isolates were A. baumannii
- 22.50% were S. pneumoniae
- 7.50% were K. pneumoniae
- 5% were E. coli
- 0.035% were Proteus spp
- 1% were S. aureus
- 0.5% were Citrobacter spp
- 0.5% were Providencia spp

Figure 1

Distribution of frequently isolated species according to the patients’ gender. Tracheal infection was found to be more prevalent in males than in females.

Figure 2
Figure 3

Percentages of total patients in each age group. The prevalence of tracheal infections was highest among patients aged 50-59 years (22%). The least prevalence rate (1%) was found in young adults aged 13-19 years.

| Age Group | Patients (%) |
|-----------|--------------|
| Baby      | 6            |
| Young Adults | 5    |
| Mid Aged Adults | 1   |
| Old Adults | 36           |
| 0-2 years  | 5            |
| 3-12 years | 1            |
| 13-19 years| 3            |
| 20-29 years| 6            |
| 30-39 years| 5            |
| 40-49 years| 12           |
| 50-59 years| 22           |
| 60-69 years| 21           |
| 70-79 years| 13           |
| 80-89 years| 6            |
| 90-99 years| 0            |

Figure 4

Prevalence of MDR and XDR isolates causing tracheal infections was showed in the pie chart. MDR isolates accounted for 68% of the infections, XDR isolates for 22%, and non-DR isolates for 10%.
Distribution of frequently isolated MDR (grey bars) and XDR (black bars) bacterial strains causing tracheal infections as 57% A. baumannii were MDR and 29% were XDR. 71.1% K. pneumoniae were MDR and 20% were XDR organisms.

![Figure 5](image)

| Strain          | MDR  | XDR |
|-----------------|------|-----|
| P. aeruginosa   | 70.49| 23  |
| A. baumannii    | 56.89| 29  |
| K. pneumoniae   | 71.1 | 20  |
| S. pneumoniae   | 80   | 7   |
| E. coli         | 50   | 20  |
| S. aureus       | 100  | 0   |

Figure 5

Distribution of β-lactamase among major bacterial strains causing tracheal infections. The prevalence of carbapenemase (black bars) was higher than ESBL (gray bars) in isolated bacteria.

![Figure 6](image)

| Strain          | Carbapenemase | ESBL |
|-----------------|---------------|------|
| A. baumannii    | 48            | 31   |
| E. coli         | 50            | 30   |
| K. pneumoniae   | 49            | 36   |
| P. aeruginosa   | 57            | 36   |
| S. pneumoniae   | 27            | 0    |

Figure 6