Research Article

β-Lactamase-Producing Multidrug-Resistant Bacterial Pathogens from Tracheal Aspirates of Intensive Care Unit Patients at National Institute of Neurological and Allied Sciences, Nepal

Santosh Khanal,1,2 Dev Raj Joshi,1 Dwij Raj Bhatta,1 Upendra Devkota,3 and Bharat Mani Pokhrel4

1 Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal
2 National College (NIST), Tribhuvan University, Kathmandu, Nepal
3 National Institute of Neurological and Allied Sciences (NINAS), Kathmandu, Nepal
4 Department of Microbiology, Institute of Medicine (IOM), Tribhuvan University, Kathmandu, Nepal

Correspondence should be addressed to Santosh Khanal; santoshkhanal007@gmail.com

Received 24 June 2013; Accepted 22 July 2013

Academic Editors: G. Alexandre, H. Asakura, and I. Morozov

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The widespread use of tracheal intubation and mechanical ventilation to support the critically ill patients increases the risk of development of tracheobronchitis and bronchopneumonia. This cross-sectional study was conducted with an aim to isolate and identify bacterial pathogens from tracheal aspirates producing extended-spectrum β-lactamase (ESBL), AmpC β-lactamase, and metallo-β-lactamase (MBL) from August 2011 to April 2012 at National Institute of Neurological and Allied Sciences (NINAS), Kathmandu, Nepal. ESBL was detected by combined disk assay using cefotaxime and cefotaxime with clavulanate, AmpC β-lactamase by inhibitor-based method using cefoxitin and phenylboronic acid, and MBL by Imipenem-EDTA combined disk method. 167 bacterial strains were isolated from 187 samples and majority of them were Acinetobacter spp. followed by Klebsiella pneumoniae with 32.9% and 25.1%, respectively. 68.8% of isolates were multidrug resistant (MDR) and Acinetobacter spp. constituted 85.4%. ESBL, AmpC β-lactamase, and MBL were detected in 35 (25%), 51 (37.2%), and 11 (36.7%) isolates, respectively. Pseudomonas spp. (42.8%) were the predominant ESBL producer while Acinetobacter spp. were the major AmpC β-lactamase producer (43.1%) and MBL producer (54.5%).

1. Introduction

Tracheostomy is a surgical procedure that creates an opening directly into the trachea to ventilate and aspirate the patient in critical care setting [1]. The incidence of ventilator-associated pneumonia (VAP) ranges from 10 to 25% of all intensive care unit (ICU) patients resulting in high mortality rate of 22–71%, which is 6–21 times higher in intubated patients [2].

The tracheostomized patients are colonized or infected with bacteria either endogenously or exogenously. Exogenous bacteria include Pseudomonas spp., Acinetobacter spp., methicillin-resistant Staphylococcus aureus (MRSA), and members of Enterobacteriaceae and endogenous bacteria include Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis. These bacteria are usually resistant to multiple antibiotics and cause either tracheobronchitis or bronchopneumonia [3]. Risk factors for colonization or infection with multidrug-resistant bacterial species include prolonged length of hospital stay, exposure to an ICU, receipt of mechanical ventilation, colonization pressure, exposure to broad-spectrum antimicrobial agents, recent surgery, invasive procedures, and underlying severity of illness [4, 5].
β-Lactamases are the commonest cause of bacterial resistance to β-lactam antimicrobial agents, which are used in the treatment of various serious infections. With the increased use of antimicrobial agents, bacteria responded with a variety of new β-lactamases including extended-spectrum β-lactamases, plasmid-mediated AmpC β-lactamases and metallo-β-lactamases [6]. Infections caused by multidrug-resistant bacteria expressing β-lactamases pose serious challenges to clinicians because these bacteria are resistant to a broad range of β-lactams, including third-generation cephalosporins, and nosocomial infections caused by these organisms complicate therapy and limit treatment options [6, 7].

The emergence and spread of antimicrobial resistance due to the production of β-lactamases as a major problem have drawn attention to a need for better diagnostic techniques and newer drugs to allow more specific therapy. Therefore, the characterization and antibiotic susceptibility pattern of β-lactamase-producing organisms can lead to successful infection control, involving antimicrobial stewardship and public health interventions aimed at controlling the emergence of such life-threatening multidrug-resistant bacteria. Hence, this study was undertaken to detect the bacterial pathogens and determine the antimicrobial resistance pattern of clinically relevant bacteria producing extended-spectrum β-lactamase, AmpC β-lactamase, and metallo-β-lactamase from tracheal aspirate of patients admitted to ICU.

2. Materials and Methods

This cross-sectional study was conducted at National Institute of Neurological and Allied Sciences, Bansbari, Kathmandu, Nepal, from August 2011 to April 2012. A total of 187 tracheal aspirate samples were included in the study.

2.1. Specimen Collection. The samples were collected in mucus trapper by applying negative pressure through automated machine by experienced physician and samples were immediately transported to the laboratory.

2.2. Culture of the Specimen. The specimens were inoculated on blood agar, MacConkey agar, and chocolate agar plates. In the chocolate agar plate, a 5 μg optochin disc and a 10 U bacitracin disc were added to screen out S. pneumoniae and H. influenzae, respectively, and the plates were incubated at 37°C overnight in candle jar, whereas, the MacConkey and blood agar plates were incubated under aerobic condition [8].

2.3. Identification and Antibiotic Susceptibility Test. The isolates were identified on the basis of colony characterization, staining, and biochemical tests such as oxidase, catalase, sulfide indole motility, citrate, urea hydrolysis, triple sugar iron agar test, and coagulase tests [8]. Antibiotic sensitivity test was performed using the Kirby-Bauer disk diffusion method and sensitivity results were interpreted according to CLSI guidelines [9]. Multidrug resistance was defined as resistance to three or more of the antimicrobial agents belonging to different structural classes [10].

| Organisms | Frequency | MDR (%) |
|-----------|-----------|---------|
| Gram-negative bacteria | 154 | 108 (70.1) |
| Acinetobacter spp. | 55 | 47 (85.4) |
| K. pneumoniae | 42 | 31 (73.8) |
| Pseudomonas spp. | 37 | 19 (51.3) |
| E. coli | 12 | 6 (50) |
| Enterobacter spp. | 3 | 2 (66.7) |
| K. oxytoca | 2 | 0 |
| Proteus vulgaris | 1 | 1 (100) |
| Gram-positive bacteria | 13 | 7 (53.8) |
| S. aureus | 12 | 6 (50) |
| S. pneumoniae | 1 | 1 (100) |
| Total | 167 | 115 (68.8) |

2.4. Test for ESBL, AmpC β-Lactamase, and MBL Production. ESBL was detected by combined disk assay using cefotaxime (30 μg) and ceftoxime (30 μg) with clavulanate (10 μg) [9]. AmpC β-lactamase by inhibitor-based method using cefoxitin (30 μg) and cefoxitin (30 μg) with phenylboronic acid (20 μL) [11], and MBL by combined disk assay using Imipenem (10 μg) and Imipenem (10 μg) with 100 mM EDTA (10 μL) [12].

3. Results

Out of 187 tracheal aspirate samples, 138 males and 49 females, 146 (78.1%) samples showed significant growth with 21 polymicrobial growth. 167 bacterial strains were identified and among them, 115 (68.8%) were multidrug resistant. Among 167 isolates, Gram-negative bacteria constituted 154 (92.2%) of the total isolates, among which 108 (70.1%) were MDR. Among Gram-negatives, Acinetobacter spp. were the most frequently isolated species with 55 (32.9%) isolates and among them, 47 (85.4%) were found to be MDR-strains. Gram-positive organisms constituted 13 (7.8%) of the total isolates and 7 (53.8%) of them were MDR. Staphylococcus aureus constituted 12 isolates and 6 (50%) of these were MDR. The results are shown in Table 1.

| Organisms         | Frequency | MDR (%) |
|-------------------|-----------|---------|
| Acinetobacter spp. | 55 | 47 (85.4) |
| K. pneumoniae     | 42 | 31 (73.8) |
| Pseudomonas spp.  | 37 | 19 (51.3) |
| E. coli           | 12 | 6 (50) |
| Enterobacter spp. | 3 | 2 (66.7) |
| K. oxytoca        | 2 | 0 |
| Proteus vulgaris  | 1 | 1 (100) |
| Gram-positive bacteria | 13 | 7 (53.8) |
| S. aureus         | 12 | 6 (50) |
| S. pneumoniae     | 1 | 1 (100) |
| Total             | 167 | 115 (68.8) |

ESBL production was confirmed in 35 (25%) isolates and the majority consisted of Pseudomonas spp. with 15 (42.8%) followed by K. pneumoniae with 12 (34.3%). Out of the 51 (37.2%) AmpC β-lactamase-positive isolates, Acinetobacter
Table 2: Antibiotic resistance rates (%) for predominant Gram-negative bacilli recovered from tracheal aspirate of ICU patients.

| Antibiotics   | Acinetobacter spp. (N = 55) | K. pneumoniae (N = 42) | Pseudomonas spp. (N = 37) | E. coli (N = 12) |
|---------------|-----------------------------|------------------------|---------------------------|-----------------|
| Amikacin      | 78.2                        | 54.8                   | 40.5                      | 8.3             |
| Ampicillin    | NT                          | 100                    | NT                        | 100             |
| Carbenicillin | NT                          | NT                     | 43.2                      | NT              |
| Cefepime      | 96.4                        | 83.3                   | 78.4                      | 66.7            |
| Cefotaxime    | 87.3                        | 78.6                   | 83.8                      | 58.3            |
| Cefoxitin     | 94.5                        | 81                     | 89.2                      | 66.7            |
| Ciprofloxacin | 80                          | 64.3                   | 51.4                      | 66.7            |
| Cotrimoxazole | 96.4                        | 85.7                   | 86.5                      | 83.3            |
| Erythromycin  | NT                          | NT                     | NT                        | 25              |
| Gentamicin    | 83.6                        | 69                     | 43.2                      | 25              |
| Imipenem      | 23.6                        | 9.5                    | 16.2                      | 0               |
| Ofloxacin     | 80                          | 64.3                   | 51.4                      | 66.7            |
| Piperacillin-tazobactam | 69.1 | 57.1 | 29.7 | NT |
| Polymyxin B   | 0                           | 0                      | 0                         | NT              |

*NT: not tested.

Table 3: Antibiotic resistance rates (%) for Gram-positive cocci recovered from tracheal aspirate of ICU patients.

| Antibiotics | S. aureus (N = 12) | S. pneumoniae (N = 1) |
|-------------|--------------------|-----------------------|
| Amikacin    | NT                 | 100                   |
| Ampicillin  | 83.3               | 100                   |
| Cefoxitin   | 33.3               | 100                   |
| Cefotaxime  | NT                 | 100                   |
| Cloxacillin | 33.3               | NT                    |
| Ciprofloxacin | 33.3             | 0                     |
| Cotrimoxazole | 30              | 100                   |
| Erythromycin | 41.7              | 100                   |
| Gentamicin  | 33.3               | 100                   |
| Methicillin | 33.3               | NT                    |
| Vancomycin  | 0                  | 0                     |

*NT: not tested.

spp. were the most frequent ones with 22 (41.5%) followed by K. pneumoniae with 13 (24.5%). MBL production was confirmed in 11 (36.7%) bacterial isolates and among them, 6 (54.5%) isolates were Acinetobacter spp. followed by K. pneumoniae and Pseudomonas spp., each with 2 (18.2%) and a single isolate of K. oxytoca (9.1%). The results are shown in Table 4.

4. Discussion

The results of the study showed high growth rate, which was in accordance with the previous study, which reported culture positivity of 90% [13]. Polymicrobial growth was observed in one-tenth of the cases and the growth of multiple organisms from tracheal specimen has been mentioned in similar studies [14, 15]. The colonization of the oropharynx, aspiration of the contaminated secretions into the lower airway, mechanical ventilation, and endotracheal tube biofilm play important role as reservoirs for infecting microorganisms [15].

In the present study, 85.4% of Acinetobacter spp., the most predominant isolate of tracheal aspirate, were MDR-strains. High level of resistance by Acinetobacter spp. was shown against cotrimoxazole (96.4%), cefotaxime (87.3%), ciprofloxacin (80%), and amikacin (78.2%). Similar trends in antimicrobial resistance (85% to ceftazidime and ciprofloxacin, 82% to cotrimoxazole, and 67% to amikacin) of Acinetobacter spp. have been observed [16]. Acinetobacter species possess a wide array of β-lactamases that hydrolyze and confer resistance to penicillins, cephalosporins, and carbapenems. The other mechanisms of resistance include loss of porin proteins and presence of multiple efflux pumps that remove wide range of antibiotics out of the bacterial cell [17].

In this study, 73.8% of K. pneumoniae were MDR-strains. These isolates showed high level of resistance against cotrimoxazole (85.7%), cefotaxime (78.6%), gentamicin (69%), and ciprofloxacin (64.3%), which was in harmony with the previous study that reported resistance of 63.1% to cotrimoxazole, 90.5% to cefotaxime, 89% to gentamicin, and 65.8% to ciprofloxacin [18]. High level of drug resistance seen among K. pneumoniae is mediated by the production of various types of β-lactamas primarily ESBL, AmpC, and metallo-β-lactamases along with drug efflux [19].

In the present study, 51.3% of Pseudomonas spp. were MDR-strains. Pseudomonas spp. were resistant to cotrimoxazole (86%), cefotaxime (83.8%), ciprofloxacin (51.4%), and gentamicin (43.2%) which was comparable to the results of two studies [18, 20]. Pseudomonas spp. display an elevated level of drug resistance mechanisms that include production of different types of β-lactamas primarily ESBL, AmpC enzymes, and metallo-carbapenemases, aminoglycoside-modifying enzymes, loss of porin proteins, and the presence of efflux pumps like MexAB-Opr M [17].

ESBL production was confirmed in 35 (25%) screen-positive isolates and the highest number of ESBL production was detected in Pseudomonas spp. (42.8%) followed by K. pneumoniae (34.3%), which was in contrary to one of
**Table 4:** Profile of \(\beta\)-lactamase-producing bacterial strains from tracheal aspirate of ICU patients.

| Organisms            | ESBL producers (no. and %) | AmpC \(\beta\)-lactamase producers (no. and %) | MBL producers (no. and %) |
|----------------------|-----------------------------|---------------------------------------------|--------------------------|
| Acinetobacter spp.   | 5 (14.3)                    | 22 (43.1)                                   | 6 (54.5)                 |
| K. pneumoniae        | 12 (34.3)                   | 13 (24.5)                                   | 2 (18.2)                 |
| Pseudomonas spp.     | 15 (42.8)                   | 9 (17.6)                                    | 2 (18.2)                 |
| E. coli              | 3 (8.6)                     | 4 (7.8)                                     | 0                        |
| Enterobacter spp.    | 0                           | 2 (4)                                       | 0                        |
| K. oxytoca           | 0                           | 1 (2)                                       | 1 (9.1)                  |
| C. freundii          | 0                           | 0                                           | 0                        |
| P. vulgaris          | 0                           | 0                                           | 0                        |
| S. aureus            | 0                           | 0                                           | 0                        |
| S. pneumoniae        | 0                           | 0                                           | 0                        |
| Total                | 35 (25)                     | 51 (37.2)                                   | 11 (36.7)                |

the studies conducted in Nepal that showed higher prevalence of *E. coli* with 80% and *K. pneumoniae* with 57.1% [21]. Higher rate of ESBL production in *P. aeruginosa* has now been increasingly reported due to predominantly occurring SHV- and OXA-type ESBLs [7].

AmpC \(\beta\)-lactamase was confirmed in 51 (37.2%) of the screen-positive isolates and *Acinetobacter* spp. constituted 22 (43.1%) followed by *K. pneumoniae* with 13 (25.5%). Plasmidic AmpC genes are derived from the chromosomal AmpC genes of *Enterobacter cloacae*, *Citrobacter freundii*, *Morganella morganii*, and *Hafnia alvei*. Most plasmid-mediated AmpC \(\beta\)-lactamases are constitutively expressed, but some enzymes, such as DHA-1, DHA-2, ACT-1, CFE-1, and CMY-13, are inducible and may be more clinically dangerous conferring the capability for an organism to become more resistant during \(\beta\)-lactam therapy [22]. In this study, phenylboronic acid was used as an inhibitor of AmpC \(\beta\)-lactamase and high sensitivity and specificity of 90% and 98.2%, respectively, for this method have been reported [11].

MBL production was confirmed in 11 (36.7%) of the screen-positive isolates that constitute 54.5% of *Acinetobacter* spp., *K. pneumoniae*, and *Pseudomonas* spp., each with 18.2% and 9.1% of *K. oxytoca*. In contrast with this finding, a Korean study reported MBL production in only 14.2% of *A. baumannii* and 11.4% of *P. aeruginosa* [23]. The most common transferable MBL families include the VIM-, IMP-, GIM-, SPM-, and SIM-type enzymes, which have been detected primarily in *P. aeruginosa* but are also found in other Gram-negative bacteria, including nonfermenters and members of the family Enterobacteriaceae [24]. MBL-producing bacteria are an increasing public health problem worldwide and mortality rates have been increased due to inadequate empirical therapy [25]. In the present study, MBL production was detected using Imipenem-EDTA combined disk method, which has sensitivity and specificity of 100% and 98%, respectively [26].

**5. Conclusion**

We conclude that Gram-negative bacilli were the predominant isolates of tracheal aspirate of ICU patients. There is a high rate of resistance to cephalosporins, fluoroquinolones, aminoglycosides, and cotrimoxazole. \(\beta\)-lactamases confer a high level of resistance to \(\beta\)-lactam antibiotics and these traits are usually carried in transferable genes, which are capable of being acquired by normally nonpathogenic bacteria. Therefore, early detection in routine laboratory, immediate infection control, and antibiotic stewardship programs should be implemented in order to limit the spread of \(\beta\)-lactamase-producing organisms.

**Acknowledgment**

The authors would like to acknowledge all the staffs of National Institute of Neurological and Allied Sciences for their cooperation.

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