Antibiotic Resistance and Sensitivity Pattern of Metallo-β-Lactamase Producing Gram-Negative Bacilli in Ventilator-Associated Pneumonia in the Intensive Care Unit of a Public Medical School Hospital in Bangladesh

Running Title: Healthcare-Associated Infections: Antibiotic Resistance and Sensitivity

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Abstract

Background: Ventilator-associated pneumonia (VAP) is the most common nosocomial infection in intensive care units (ICU), which accounts for 25% of all ICU infections. The development of resistance to antimicrobials is increasing and becoming a significant health problem worldwide increasing morbidity, mortality and costs. In hospitals, this adds to length of stay as well as mortality. Identifying the antibiotic resistance pattern in VAP is important as this can cause outbreaks in ICUs, responsible for increased mortality and morbidity or limiting therapeutic options. To date, there have been limited studies assessing this in Bangladesh. Consequently, the primary objective of this research project was to study the species of bacterial growth and to determine the antibiotic resistance patterns of Metallo-β-Lactamase (MBL) producing gram-negative bacilli among ICU patients with VAP in a public medical school Hospital, Bangladesh. In addition, identify the factors associated with a positive culture to provide future guidance. ***Method***: Cross-sectional study performed in the Chattogram Medical

College Hospital, Bangladesh. Mueller Hinton agar plates were used for antibiotic sensitivity testing by the Kirby-Buer disc diffusion test. ***Results***: Among the 105 clinically suspected VAP cases, qualitative cultures were positive in 95 (90%) of them. The most common bacteria identified were *Acinetobacter spp*. (43.2%), *Klebsiella spp. (20%)* and *Pseudomonas spp. (18.9%)*. A positive culture was not associated with patients' age or gender. Among the 41 isolated *Acinetobacter spp.*, 38 (92.7%) were resistant to gentamicin followed by 36 (87.8%) to ceftriaxone. Among the 24 isolated *Klebsiella spp*., 22 (83.3%) were resistant to ceftriaxone. Among 18 isolated *Pseudomonas spp*., 16 (88.8%) were resistant to ciprofloxacin, and 13 (72.2%) were resistant to ceftriaxone. Among nine isolated E. coli, all were resistant to ceftriaxone and ciprofloxacin. All four *Proteus spp.* (100%) isolated were resistant to ciprofloxacin. Additionally, phenotype MBL producing was 65.22% and Genotype was 45.65% among imipenem resistant pathogens. Imipenem resistant pathogens were sensitive to amoxyclav, amikacin¸ azithromycin, ceftazidime, ceftriaxone, colistin and gentamycin ***Conclusion***: A positive culture was detected in 90% of VAP patients, but it was not associated with the patients' age and gender. The most common bacteria identified as *Acinetobacter spp*., *Klebsiella spp.* and *Pseudomonas spp.,* where the majority of these were resistant to ceftriaxone. The results are being used to provide guidance on the empiric management of VAP in the future in this hospital.

Keywords: HCAIs, Healthcare-Associated Infections, Antibiotic Resistance, Intensive Care Unit, Public Medical School Hospital, Bangladesh.

1. INTRODUCTION

Healthcare-associated infections (HAIs) are infections that occur whilst patients are receiving health care in a hospital or other health care facility that first appear 48 hours or more after hospital admission, or within 30 days after having received health care (1). Nosocomial infections, or else known as hospital-acquired infections, are the synonym of HAIs (1, 2). There are six different broad categories of HAIs, namely healthcare-associated pneumonia, urinary tract infection, surgical site infections, *Clostridium difficile* infections, neonatal sepsis, and primary bloodstream infections (3). Other studies though have reported four wide-ranging types of HAIs including catheter-associated bloodstream infections (CABSIs) or central line-associated bloodstream infections (CLABSIs), catheter-associated urinary tract infections (CAUTIs), surgical site infections (SSIs), and ventilator-associated pneumonia (VAP) (1, 4, 5).

HAIs are becoming an increasing global public health concern including South Asian countries as they can increase morbidity, mortality, and costs (1, 6-11). CABSIs appear the most costly at $45,814 per case in the US, followed by VAP at $40,144,per case and SSIs at $20,785 per case (12). Costs are enhanced in the US with median length of stays approximately 2-fold higher in patients with HAIs compared with those without HAIs (13). Overall, HAIs affect the health status of hospital patients in both high- as well as lower- and middle-income countries (6, 7, 14, 15). However, HAIs appear appreciably more common in LMICs averaging up to 8% to 19% or higher among hospital patients (7, 16-23). This situation in LMICs is compounded by a number of countries lacking effective infection prevention and control (IPC) programs exacerbated by the lack of antibiotic policies, poor laboratory support, limited resources, suboptimal adherence to safe practices and typically limited compulsion to report HAIs (21, 24-26). However, we are beginning to see a number of studies undertaken in LMICs to document ongoing initiatives to reduce HAI rates including educational initiatives, instigating infection prevention control groups and hand hygiene initiatives, monitoring the prescribing of antibiotic prophylaxis to prevent SSIs and initiating programs to reduce VAP including sedation and weaning protocols as well as mechanical ventilation protocols (27-33). Regular surveillance of HAIs is also crucial as part of agreed future strategies to

reduce HAIs within hospitals (19, 34). Such strategies are particularly important in LMICs already experiencing high rates of AMR such as Bangladesh (35-38).

VAP is defined as pneumonia that arises 48-72 hours or subsequently following endotracheal intubation, categorized by the existence of a new or progressive infiltrate, signs of systemic infection, such as fever, increased white blood cell count, changes in sputum features, and recognition of contributing microorganisms (39-41). VAP is the most common HAI in the intensive care unit (ICU), which accounts for 8-38% of all ICU infections. VAP often increases hospital and ICU stay with an accompanying increase in costs, and is associated with higher morbidity and mortality (39-46), with the incidence of VAP typically 3 to 10 times higher in ICU than among patients on general wards (47-52). In additional, VAP related mortality has been reported to be 24-76% higher than other HAIs (53). Early detection of the causative pathogens and antimicrobial sensitivity patterns are the key issues to promote and ensure better treatment among patients diagnosed with VAP (54, 55). Additionally, the requirement to precisely identify VAP is critical so that antimicrobials can be stopped at the optimal time to decrease their unnecessary use and any associated AMR (56). The irrational use of antibiotics in ICU often increases adverse drug reactions and healthcare costs alongside promoting AMR, with the instigation of rapid diagnostic tests in ICUs helping to reduce the over use of especially broad spectrum antibiotics (57-59).

Lower respiratory tract samples obtained either by invasive [Protected specimen brush (PSB) or Broncho-alveolar lavage (BAL)] or noninvasive [Endotracheal aspiration (ETA)] techniques have been used to identify causative pathogens related to VAP (60, 61). Gram staining, qualitative and semi-quantitative culture of ETA necessitates petite methodological know-how but no dedicated apparatus (62). Quantitative ETA culture is a non-invasive technique, with technicians rapidly acquiring the necessary skills, and low-priced compared with quantitative BAL fluid cultures. In addition, studies have recommended that ETA can be used as a substitute for BAL in quantitative cultures (60, 63). The optimum values considered for establishing pneumonia by quantitative cultures are $\geq 10^5$ to 10^6, $\geq 10^4$ and 10^3 CFU/ml for quantitative cultures of ETAs (QEA), bronchoscopic BAL and PSB correspondingly with 10^5 CFU/ml being the most broadly recognized value for QEA (53, 64, 65).

The emergence of resistance to antimicrobial agents is becoming a significant health problem worldwide, especially in HAIs (1, 6, 21, 66-68). A study from China reported that participants largely had a high level of understanding regarding hand hygiene, the core concept of HAIs, and healthcare worker safety; however, a low level of knowledge about HAI's pathogenic microorganism documentation and isolation issues (69). This is important since for instance if patients in ICU receive appropriate therapy according to the microbiology results their outcomes are improved (70). A study conducted in Yemen revealed variable knowledge and practice regarding HAIs among nurses, which could be improved after educational interventions (71). Other studies have also shown variable knowledge about HAIs among healthcare workers (72, 73). Whilst health professionals in a Vietnamese hospital were generally reasonably well-informed regarding theories surrounding HAIs, they were typically not well informed and conscious about their own hospital and infection control policy and plans with practices to prevent HAIs typically poor (74).

A first step to reducing the morbidity and mortality associated with VAP in ICUs is to document current antibiotic resistance patterns in these patients to help develop and refine future prevention and treatment strategies, which could include future empiric antibiotic prescribing guidance (75). Documenting such patterns in ICU patients with VAP helps pro-actively refine prescribing practices to

reduce future morbidity and mortality (75-79). This includes the extent of Metallo-β-Lactamase Producing Gram-Negative Bacilli (MBL).

There are several different types of MBL producing bacilli have identified, which include Imipenemase (IMP), Verona integron-encoded metallo-β-lactamase (VIM), Sao Paulo metallo-β-lactamase (SPM), Germany imipenemase (GIM), New Delhi metallo-β-lactamase (NDM), Florence imipenemase (FIM) and Seoul imipenemase (SIM)variants (80, 81) with IMP and VIM the most predominant (82). Acquired MBL has in recent times appeared as one of the most troublesome resistance concerns because of their capability to hydrolyze all β-lactams, including carbapenems. MBL producing pathogens strains are not sensitive to serine β-lacatamase inhibitors such as clavulanate and sulfones and MBL genes have the capacity to spread because of their extreme transportable potential (83). Moreover, MBL resistance results in higher morbidity and mortality as well as increasing hospital stay and costs (84, 85). Multiple studies have reported that 74%-96.6% of *A. baumannii* were MBL producing (86-88). Another Iranian study revealed that 55% of clinically isolated *P. aeruginosa* were resistant to imipenem and meropenem, among which 37.72% were the MBL producers (88). Presently, the most extensively utilized cataloguing system for β-lactamases is the Ambler operational classification, which is constructed on sequence resemblance, and separates β-lactamases into 4 classes: the classes A, C, and D of serine-β-lactamases (SBLs) and the class B of MBLs (89-91). MBL detection is usually conducted utilizing ethylene diamine tetra acetic acid (EDTA) as the MBL inhibitor. Furthermore, four phenotypic methods are commonly utilized. Those are **(i)** Combined disk synergy test (CDST) with 0.5M EDTA (CDST-0.5 M EDTA), (**ii)** CDST with 0.1 M EDTA (CDST-0.1 M EDTA), (**iii**) (3) double-disk synergy test (DDST) with 0.5M EDTA (DDST-0.5 M EDTA), and (**iv**) DDST with 0.1 M EDTA (DDST-0.1 M EDTA) (92). DNA extraction and multiplex PCR amplification are commonly used for the simultaneous detection of NDM, VIM, and IMP MBL genes (93). Generally, the aztreonam-ceftazidime-avibactam combination has the greatest effect for the treatment of MBL producing infectious diseases versus aztreonam-amoxicillin-clavulanate and aztreonam -ceftolozane-tazobactam (94). However, on several occasions aztreonam-amoxicillin-clavulanate has been found to be as effective as aztreonam-ceftazidime-avibactam (94). Importantly for LMICs, the aztreonam-amoxicillin-clavulanate combination is also currently cheaper than other combinations (94).

Consequently, this study was designed to explore the species of bacterial growth and the antibiotic resistance among ICU patients with VAP in a leading public medical school Hospital in Bangladesh as well as possible factors associated with a positive culture. This study results will help provide guidance to managing patients in this ICU with VAP as well as other ICUs in Bangladesh and wider dealing with similar patients.

2. MATERIALS and METHODS

2.1 *Study Design and Sample*

This is a cross-sectional study whereby the data collection was performed only once. All patients who were on mechanical ventilation by endotracheal tube for more than 48 hours along with two or three of the following criteria suggesting VAP: (i) fever/hypothermia or leukocytosis / leucopenia; (ii) purulent tracheal discharge; or a (iii) positive chest X-ray (chest X-ray shows consolidation or infiltration or pleural effusion) were included in the study (95). Exclusion criteria included patients who had severe hypoxemia (PaO_2/FiO_2<100), were immune-compromised or neutropenic (77).

Overall, a total of 105 suspected VAP ICU patients age ranging from 16-92 years admitted to Chattogram Medical College Hospital in Bangladesh between July-2017 to June-2018 were included in this study. This study was part of an overall project assessing the extent of MBL producing gram-negative organisms among VAP patients in the ICU in this hospital (75). Several antibiotics were evaluated against isolated organisms. These included amoxiclav, amikacin, azithromycin, ceftazidime, ceftriaxone, colistin, ciprofloxacin, gentamicin, imipenem, levofloxacin, oxacillin, pipercillin-tazobactam and vancomycin. Bio-Rad™ ASD was utilized in this study to assess sensitivity patterns. MBL producing gram-negative bacteria was detected through a phenotype method by CDST and genotype by multiplex PSR (75). Researchers did not perform any tests to assess AZTREONAM resistance and sensitivity patterns due to financial limitations within the hospital.

2.2 Data Collection and Analysis

Data was collected, recorded, edited, and analyzed in a predesigned datasheet. A spreadsheet of Microsoft Xcel was developed to keep the record of all necessary data that was utilized for data analysis.

Standard methods were used for the analysis and culture of ETA specimens collected from all suspected patients (60). Immediately after receipt, one ml of ETA was diluted to a final concentration of 1:100 in sterile Phosphate-buffered saline (PBS). After being manually stirred, a sample was taken with the help of 0.01 ml calibrated loop and cultured on 5% sheep blood agar, chocolate agar, and MacConkey's agar plates, and subsequently incubated 24-48 hours. Anaerobic organisms were incubated in a 5% CO_2 at 35°C for 48-72 hours. All isolates were identified based on their colony morphology, culture characteristics, and biochemical reactions according to the standard microbiological procedures. Mueller Hinton agar plates were used for antibiotic sensitivity testing by disc diffusion test. Bacterial isolates were tested for antimicrobial sensitivity by the Kirby Bauer disc diffusion technique (60, 61).

2.3 Statistical Analysis

The results of the experiments were recorded and analyzed systematically using descriptive analysis (frequency and percentage) for the positive culture growth and the resistance towards the antibiotics tested. The factors associated with positive culture growth from the samples taken, namely age and gender, were analyzed using simple and multiple logistic regression where the significance level was set at 0.05 for 95% confidence interval (CI), using SPSS software (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. IBM Corp.: Armonk, New York, NY, USA).

2.4 Ethical Approval

This study obtained ethical approval from the Institutional Review Board of Chattogram Medical College, Chattogram, Bangladesh (Reference No.: CMC/PG/2017/322, Date: 04-05-2017).

3. RESULTS

Phenotype MBL producing was 65.22% and Genotype (Multiplex PSR) was found 45.65%. Approximately two-thirds of the 105 suspected VAP patients were males (n=72, 68.6%). The mean age of the 105 patients was 47.8 years old (standard deviation = 21.17) with the minimum and maximum age of 16 and 92 years old, respectively. Ninety percent (n=95) of the total clinically suspected VAP patients were found to be positive for the quantitative culture method. The distribution of positive and negative culture according to the gender of patients is shown in Table 1. Further testing performed showed that the identified organisms were *Acinetobacter* spp. (43.2%), *Klebsiella spp.* (20%), *Pseudomonas* spp. (18.9%), *E. coli* (8.9%), CoN *Staphylococcus* (2.2%), *Staphylococcus aureus* (2.2%) and mixed microbial growth (7.8%) (Table 2).

Table 1. Distribution of positive and negative bacterial culture according to the gender of patients (n=105).

	Male patients		Female patients		TOTAL	
Culture	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
Positive	67	93.1%	28	84.8%	95	90.5%
Negative	5	6.9%	5	15.2%	10	9.5%

Table 2. Distribution of Bacteria Isolated by Quantitative Culture (n=95).

Bacteria	Number	Percentage (%)
Acinetobacter spp.	41	43.2
Klebsiella spp.	18	20
Pseudomonas spp.	17	18.9
E.coli	8	8.9
CoN *Staphylococcus*	2	2.2
Staphylococcus aureus	2	2.2
Mixed growth	7	7.8

Normally, the trachea is a sterile site like cerebrospinal fluid (CSF), blood, peritoneal fluid, and pleural fluid. Thereafter, microbial growth is unexpected and indicates infection when this occurs. Consequently, as soon as multiple microorganisms are identified by quantitative culture through single Endotracheal Aspirate (ETA), which is a mixed growth of pathogens, this is considered a grave clinical situation (60).

The mixed growths in our study were *Pseudomonas* and *E. coli* (n=1), *Proteus,* and *Klebsiella* (n=4) as well as *Klebsiella* and Coagulase Negative Staphylococcus species [CoNS] (n=2). Further analysis using simple and multiple logistic regression found that the growth of bacteria from the culture of samples from the VAP patients was not associated with the patients' ages or gender (Table 3).

Table 3. Factors associated with bacteria growth from the culture of samples from ventilated-associated pneumonia (VAP) patients using simple and multiple logistic regression (n=105).

Characteristics of VAP patients	Simple Logistic Regression		Multiple Logistic Regression	
	OR (95% CI of OR)	*p*-value	OR (95% CI of OR)	*p*-value
Age	0.990 (0.961, 1.021)	0.529	0.992 (0.962, 1.022)	0.586
Gender				
Male*	1.000	-	1.000	-
Female	0.418 (0.112, 1.558)	0.194	0.412 (0.110, 1.547)	0.189

OR = Odds Ratio; CI = Confidence Interval; *reference group.

Further laboratory testing showed that among the 41 isolated *Acinetobacter* spp., 38 (92.7%) were resistant to gentamicin followed by 36 (87.8%) to ceftriaxone, 34 (82.9%) to ciprofloxacin and amoxiclav, and 33 (80.5%) to ceftazidime and piperacillin-tazobactam with only eight resistant (19.51%) to colistin

(Table 3). On the other hand, among the 24 isolated *Klebsiella* spp., including six which came from the mixed growth, 22 (91.67%) were resistant to ceftriaxone, 18 (75%) were resistant to amoxiclav, 21 (87.5%) to ciprofloxacin, 16 (66.7%) to azithromycin, and 13 (54.2%) to both gentamicin and imipenem (Table 4) Furthermore, among 18 isolated *Pseudomonas spp.* including one from the mixed growth, 16 (88.8%) were resistant to ciprofloxacin, 15 (83.3%) were resistant to ceftriaxone, gentamicin and azithromycin, and 11 (64.8%) were resistant to ceftazidime (Table 4). Among nine isolated *E. coli* including one from the mixed growth, all were resistant to ceftriaxone and ciprofloxacin followed by eight (89%) resistant to ceftazidime and seven (77.8%) to amoxiclav (Table 4). The current study also showed that from the four *Proteus spp*. which all were isolated from the mixed growth, all (100%) were resistant to ciprofloxacin and amoxiclav, three (75%) to Colistin, two (50%) to ceftriaxone, gentamicin, and azithromycin. However, all four *Proteus spp*. isolates were sensitive to amikacin. The two isolated *Staphylococcus aurei* identified were resistant to azithromycin, ciprofloxacin, and levofloxacin. Among the four CoNS, including two from mixed growth, all were resistant to ciprofloxacin (Table 4). Imipenem resistant pathogens organism were sensitive to amoxyclav, amikacin¸ azithromycin, ceftazidime, ceftriaxone, colistin and gentamycin. The details of sensitivity pattern of imipenem resistant pathogens was depicted in Table 5 and Figure 1.

Table 4. Antibiotic Resistance Patterns of Isolated Bacteria using the Kirby-Buer Disc Diffusion Method (n=102*).

Antibiotic Disc	*Acinetobacter* n=41 No. (%)	*Klebsiella* n=18 + 6** No. (%)	*Pseudomonas* n=17 + 1** No. (%)	*E. Coli* n=8 + 1** No. (%)	Proteus n=4** No. (%)	*S. Aureus* n=2 No. (%)	CoNS*** n=2 + 2** No. (%)
Amoxyclav	34 (82.9)	18 (75.0)	14 (77.7)	7 (77.8)	4 (100.0)	0	2 (50.0)
Amikacin	29 (70.7)	12 (50.0)	9 (52.9)	4 (44.4)	0	0	1 (25.0)
Azithromycin	30 (73.2)	16 (66.7)	13 (72.2)	4 (44.4)	2 (50.0)	2 (100.0)	3 (75.0)
Ceftazidime	33 (80.5)	12 (50.0)	11 (61.1)	8 (89.0)	0	0	0
Ceftriaxone	36 (87.8)	22 (83.3)	13 (72.2)	9 (100.0)	2 (50.0)	1 (50.0)	3 (75.0)
Colistin	8 (19.5)	0	0	0	3 (75.0)	-	-
Ciprofloxacin	34 (82.9)	21 (87.5)	16 (88.8)	9 (100.0)	4 (100.0)	2 (100.0)	3 (100.0)
Gentamicin	38 (92.7)	13 (54.2)	11 (61.1)	5 (55.6)	2 (50.0)	0	1 (25.0)
Imipenem	23 (56.1)	13 (54.2)	6 (33.3)	3 (33.3)	1 (25.0)	-	-
Levofloxacin	-	-	-	-	-	2 (100.0)	2 (50.0)
Vancomycin	-	-	-	-	-	0	0
Oxacillin	-	-	-	-	-	1 (50.0)	1 (25.0)
Piperacillin-Tazobactum	30 (73.2)	2 (11.1)	4 (23.5)	6 (67.0)	-	-	-

*Includes 95 single isolates and 7 mixed growth; **from diverse growth; 0=no resistance; - the antibiotics were not tested against this organism,
***CoNS = Coagulase Negative *Staphylococcus* species .

Table 5: Antibiotic Sensitivity Pattern Imipenem Resistant Pathogens

Antibiotic	Acinetobacter[¶]	Klebsiella *	Pseudomonas[¥]	E. Coli [±]
Amoxyclav	8(34.78%)	6(42.83%)	2(28.57%)	1(50%)
Amikacin	7(30.48%)	5(35.71%)	2(28.57%)	0
Azithromycin	4(17.39%)	3(21.42%)	1(14.28%)	0
Ceftazidime	6(26.08%)	2(14.28%)	3(42.83%)	1(50%)
Ceftriaxone	5(21.73%)	2(14.28%)	2(28.57%)	1(50%)
Colistin	15(65.21%)	14(100%)	7(100%)	2(100%)
Gentamycin	2(8.69%)	3(21.42%)	2(28.57%)	0

Out of total 105 cases 46 were Imipenem resistant. [¶] Acinetobacter 23. [*]Klebsiella 14. [¥]Pseudomonas 7. [±]E. Coli 2.

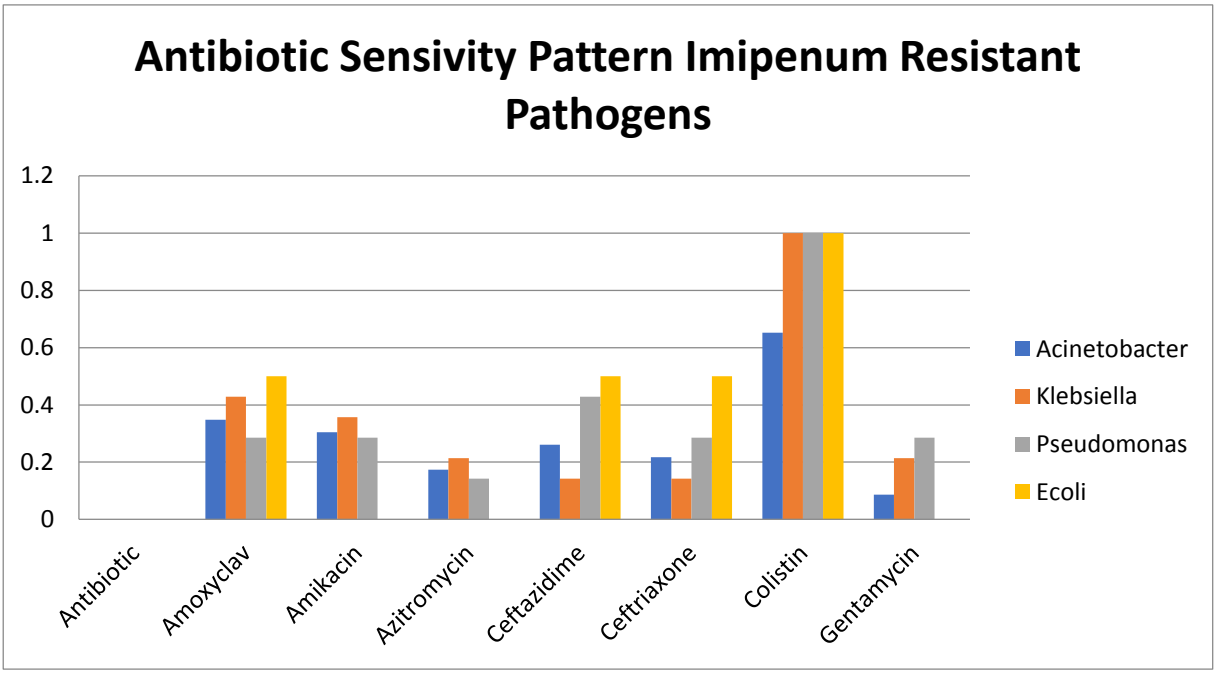

Figure 1: Illustrating the Sensitivity Pattern of Imipenem Resistant Pathogens.

4. DISCUSSION

90% of cases in our study were found culture positive by quantitative culture from 105 suspected VAP cases like other published studies (52, 53, 96-99). We found VAP principally caused *Acinetobacter spp.* as the predominant organism followed by *Klebsiella spp.*, *Pseudomonas spp.* and *E. coli*, similar to earlier studies (100, 101). Among the 41 isolated *Acinetobacter spp*. were resistant to gentamicin (92.68%); ceftriaxone (87.80%), ciprofloxacin and amoxyclav (82.93%,); ceftazidime and piperacillin-tazobactam (80.49%); azithromycin (73.17%); imipenem (56.10%); and colistin (19.51%). This is similar to another study from Turkey which found resistance *A. baumannii* to the following antibiotics: ciprofloxacin (97.3%), ceftazidime (96.8%), levofloxacin (95.2%), cefepime (93.7%), meropenem (90.3%), imipenem (89.1%), cefoperazone-sulbactam (79%), gentamicin (77.2%), trimethoprim-sulfamethoxazole (68.9%), tigecycline (41.3%), amikacin (35.2%), netilmicin (19.5%), and colistin (5.5%) (100). One study in Bangladesh similarly found a high rate of ceftriaxone (99.6%), and gentamicin (99.3%) resistance (101). In another study undertaken in Bangladesh, the authors reported that the isolates of *Acinetobacter spp.* were resistant to ciprofloxacin and ceftazidime (80%) as well as piperacillin-tazobactam (80%), and imipenem (60%) (102).

Colistin was found most effective against (80.5%) *Acinetobacter spp*. in our study, with one Indian study demonstrating that colistin was 100% effective against *Acinetobacter spp.* (103). This may not always be the case with other studies conducted in Bangladesh reported that colistin resistance mcr-1 gene has been found both in hospitalized patients urban settings (104, 105). Concerns with inappropriate use of colistin with resultant implications for antibiotic treatment of

last resort have already resulted in LMICs restricting its use, and this is likely to grow (106). The current study also revealed that *Klebsiella spp.* were resistant to ceftriaxone (91.7%), ciprofloxacin (87.5%,), amoxiclav (75.0%), azithromycin (66.7%,), both gentamicin and imipenem (54.2%), and amikacin (50.0%). This is similar to a study undertaking in Iran regarding HAIs where ceftriaxone (92%), ciprofloxacin (82%), nitrofurantoin (80%), ofloxacin (75%), cefotaxime (70%), imipenem (67%), ticarcillin (66%), nalidixic acid (60%), gentamicin (52%), azithromycin (40%), cefepime (31%), polymyxin B (22%), colistin (17%), amikacin (7%), meropenem (1%) were resistant to *Klebsiella spp*. (107). *Klebsiella spp.* were similarly resistant to ceftriaxone (95.08%), ciprofloxacin (93.2%), gentamicin (60%), imipenem (54.8%), and amikacin (50%) in other studies undertaken in Bangladesh (101, 102). Amikacin was found to be most effective against *Klebsiella spp*. in this study, similar to one Indian study (108). Among 18 isolated *Pseudomonas spp.* were resistant to ciprofloxacin (88.8%), ceftriaxone (83.33%), as well as gentamicin and azithromycin (83.33%), ceftazidime (64.77%), amikacin (52.94%), and piperacillin-tazobactam (23.53%) in the current study. Earlier studies conducted in Bangladesh revealed that *Pseudomonas spp.* were resistant to ciprofloxacin (92.8%), ceftriaxone (90.4%), ceftazidime (72.7%), amikacin (64.28%), and piperacillin-tazobactam (22.9%), similar again to our study (101, 102). Piperacillin-Tazobactum was also found to be most effective against *Pseudomonas spp.* in our study, with 76.47% sensitive. *E. coli* were resistant to ceftriaxone and ciprofloxacin (100%), ceftazidime (89%), amoxiclav (77.77%), piperacillin-tazobactam (67%), gentamicin (55.55%), and amikacin and azithromycin (44.44%). An earlier study undertaken in Bangladesh had similar findings of resistance patterns of *Escherichia coli* to ciprofloxacin (96.4%), ceftazidime (96.2%), gentamicin (62.9%), piperacillin-tazobactam (64.2%), and amikacin (44.4%), (101). In the current study *Proteus spp*. were resistant to ciprofloxacin and amoxiclav (100%), colistin, and ceftriaxone (75%), gentamicin, and azithromycin (50%). Nevertheless, *Pseudomonas spp.* isolates were sensitive to amikacin. Another study undertaken in Bangladesh also found that *Proteus spp*. isolates were resistant to ciprofloxacin and sensitive to amikacin (102).

The current study revealed that *Staphylococcus Aureus* was resistant to azithromycin, ciprofloxacin, and levofloxacin. The four CoNS isolates were also resistant to ciprofloxacin. However, an earlier study undertaken in Bangladesh revealed that *Staphylococcus Aureus* were resistant to ciprofloxacin (83.3%) and levofloxacin (66.4%) disagreeing with our findings (102). We are not sure of the reasons for this; however, this may represent growing rates of AMR in Bangladesh. In addition, earlier studies have reported that the aging process appears to act as an independent risk factor for the development of VAP in ICUs (109-112), which differs from our findings (Table 2). Liu *et al* (2017) also reported that every one-year increase age significantly correlates with an increase in the possibility of acquiring VAP by approximately 1.5-fold (111). Earlier studies also suggested that gender had a correlation with developing VAP in ICU settings, and male patients were predominantly the sufferers (45, 112, 113). We again did not find this in our study. We are not sure of the reasons for these differences and will be investigating this further.

There is currently no antimicrobial stewardship programs (ASPs) in this and other similar hospitals in Bangladesh to guide future activities especially around activities to reduce HAI rates (74, 114). This is similar to hospitals in other LMICs (115-117) and needs to be urgently addressed given rising AMR rates in Bangladesh through increasing resources for this activity taking account of local cultural issues (14, 35, 118, 119). There is also a concern that pharmaceutical companies currently appear to be heavily involved in educating physicians in this and other hospitals in Bangladesh, similar to a number of other LMICs (120-125). This again needs to be urgently addressed via active Drugs and Therapeutic Committees to provide unbiased information to hospital physicians, with key groups including microbiology teams, physicians, and pharmacists, coming together to construct robust and hospital specific treatment guidelines for key infections based on current sensitivity patterns whilst physicians wait for any culture and sensitivity tests (126-129). Subsequently, monitoring prescribing against agreed guidance championed via inter-disciplinary teams (118, 130). We will be monitoring this in the future acknowledging the difficulties with instigating ASPs and Drug and Therapeutics Committee (DTCs) in LMICs.

5. LIMITATIONS

We are aware of several limitations with this study. Firstly, we did not perform antibiotic sensitivity tests using the dilution method. This is because this test is time-consuming to perform and very labor-intensive. There are also concerns with its accuracy with considerable opportunities for mistakes in the preparation of the various antibiotic solutions. In addition, the need for comparatively large amount of reagents and space for each test, as well as the difficulties in testing in large numbers strains for multiple antibiotics, precluded this method in countries such as Bangladesh with its limited resources (131). This also applied to any assessment of resistance to AZTREONAM. Secondly, we are aware that we conducted this study in only one public medical school hospital. However, we believe our findings are robust providing direction to this hospital and wider on ways to reduce the prevalence, morbidity and mortality due to VAP.

6. CONCLUSION and RECOMMENDATIONS

A positive culture was detected in 90% of the VAP patients, but it was not associated with the patients' age and gender. The most common bacteria identified were *Acinetobacter spp.*, *Klebsiella spp.* and *Pseudomonas spp.*, where more than three-quarters of these were resistant to ceftriaxone. We believe our findings can be used to develop future guidelines to improve the prevention and management of VAP in ICU patients in this hospital through the instigation of active DTCs and ASPs in this hospital. This includes monitoring the prescribing of colistin due to fears of resistance development in Bangladesh. We will be monitoring suggested activities in the future acknowledging though the difficulties with instigating ASPs and DTCs in LMICs.

7. DISCLOSURE and FUNDING

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REFERENCES

1. Haque M, Sartelli M, McKimm J, Abu Bakar M. Health care-associated infections - an overview. Infection and drug resistance. 2018;11:2321-33.
2. Revelas A. Healthcare - associated infections: A public health problem. Niger Med J. 2012;53(2):59-64.
3. Cassini A, Plachouras D, Eckmanns T, Abu Sin M, Blank H-P, Ducomble T, et al. Burden of Six Healthcare-Associated Infections on European Population Health: Estimating Incidence-Based Disability-Adjusted Life Years through a Population Prevalence-Based Modelling Study. PLoS medicine. 2016;13(10):e1002150-e.
4. Umscheid CA, Mitchell MD, Doshi JA, Agarwal R, Williams K, Brennan PJ. Estimating the proportion of healthcare-associated infections that are reasonably preventable and the related mortality and costs. Infection control and hospital epidemiology. 2011;32(2):101-14.
5. Al-Tawfiq JA, Tambyah PA. Healthcare associated infections (HAI) perspectives. Journal of infection and public health. 2014;7(4):339-44.
6. Saleem Z, Godman B, Hassali MA, Hashmi FK, Azhar F, Rehman IU. Point prevalence surveys of health-care-associated infections: a systematic review. Pathogens and global health. 2019;113(4):191-205.
7. Allegranzi B, Bagheri Nejad S, Combescure C, Graafmans W, Attar H, Donaldson L, et al. Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. Lancet. 2011;377(9761):228-41.
8. Manoukian S, Stewart S, Dancer S, Graves N, Mason H, McFarland A, et al. Estimating excess length of stay due to healthcare-associated infections: a systematic review and meta-analysis of statistical methodology. J Hosp Infect. 2018;100(2):222-35.
9. Irek EO, Amupitan AA, Obadare TO, Aboderin AO. A systematic review of healthcare-associated infections in Africa: An antimicrobial resistance perspective. African journal of laboratory medicine. 2018;7(2):796.
10. Mythri H, Kashinath K. Nosocomial infections in patients admitted in intensive care unit of a tertiary health center, India. Ann Med Health Sci Res. 2014;4(5):738-41.
11. Choudhuri AH, Chakravarty M, Uppal R. Epidemiology and characteristics of nosocomial infections in critically ill patients in a tertiary care Intensive Care Unit of Northern India. Saudi journal of anaesthesia. 2017;11(4):402-7.
12. Zimlichman E, Henderson D, Tamir O, Franz C, Song P, Yamin CK, et al. Health care-associated infections: a meta-analysis of costs and financial impact on the US health care system. JAMA Intern Med. 2013;173(22):2039-46.

13. Glance LG, Stone PW, Mukamel DB, Dick AW. Increases in mortality, length of stay, and cost associated with hospital-acquired infections in trauma patients. Archives of surgery. 2011;146(7):794-801.
14. Khan HA, Baig FK, Mehboob R. Nosocomial infections: Epidemiology, prevention, control, and surveillance. Asian Pacific J Tropical Biomedicine. 2017; 7(5):478-482. .
15. Russo PL, Stewardson AJ, Cheng AC, Bucknall T, Mitchell BG. The prevalence of healthcare associated infections among adult inpatients at nineteen large Australian acute-care public hospitals: a point prevalence survey. Antimicrobial resistance and infection control. 2019;8:114-.
16. Phu VD, Wertheim HF, Larsson M, Nadjm B, Dinh QD, Nilsson LE, et al. Burden of Hospital Acquired Infections and Antimicrobial Use in Vietnamese Adult Intensive Care Units. PloS one. 2016;11(1):e0147544.
17. Esen S, Leblebicioglu H. Prevalence of nosocomial infections at intensive care units in Turkey: a multicentre 1-day point prevalence study. Scandinavian journal of infectious diseases. 2004;36(2):144-8.
18. WHO. Report on the Burden of Endemic Health Care-Associated Infection Worldwide. Cleaner care is safer care. Availavle at URL: http://apps.who.int/iris/bitstream/handle/10665/80135/?sequence=1.
19. Saleem Z, Hassali MA, Godman B, Hashmi FK, Saleem F. A multicenter point prevalence survey of healthcare-associated infections in Pakistan: Findings and implications. American journal of infection control. 2019;47(4):421-4.
20. Versporten A, Zarb P, Caniaux I, Gros MF, Drapier N, Miller M, et al. Antimicrobial consumption and resistance in adult hospital inpatients in 53 countries: results of an internet-based global point prevalence survey. The Lancet Global health. 2018;6(6):e619-e29.
21. Mpinda-Joseph P, Anand Paramadhas BD, Reyes G, Maruatona MB, Chise M, Monokwane-Thupiso BB, et al. Healthcare-associated infections including neonatal bloodstream infections in a leading tertiary hospital in Botswana. Hospital practice. 2019;47(4):203-10.
22. Rothe C, Schlaich C, Thompson S. Healthcare-associated infections in sub-Saharan Africa. J Hosp Infect. 2013;85(4):257-67.
23. Greco D, Magombe I. Hospital acquired infections in a large north Ugandan hospital. J Prev Med Hyg. 2011;52(2):55-8.
24. Yallew WW, Kumie A, Yehuala FM. Point prevalence of hospital-acquired infections in two teaching hospitals of Amhara region in Ethiopia. Drug Healthc Patient Saf. 2016;8:71-6.
25. Biswal M, Mewara A, Appannanavar SB, Taneja N. Mandatory public reporting of healthcare-associated infections in developed countries: how can developing countries follow? J Hosp Infect. 2015;90(1):12-4.
26. Samuel SO, Kayode OO, Musa OI, Nwigwe GC, Aboderin AO, Salami TAT, aiwo SS. Nosocomial infections and the challenges of control in developing countries. Afr J Clin Exp Microbiol. 2010;11(2):102–110.
27. Safdar N, Abad C. Educational interventions for prevention of healthcare-associated infection: a systematic review. Critical care medicine. 2008;36(3):933-40.

28. Schmier JK, Hulme-Lowe CK, Semenova S, Klenk JA, DeLeo PC, Sedlak R, et al. Estimated hospital costs associated with preventable health care-associated infections if health care antiseptic products were unavailable. ClinicoEconomics and Outcomes Research. 2016;8:197-205.
29. Mauger B, Marbella A, Pines E, Chopra R, Black ER, Aronson N. Implementing quality improvement strategies to reduce healthcare-associated infections: A systematic review. American journal of infection control. 2014;42(10 Suppl):S274-83.
30. Keyt H, Faverio P, Restrepo MI. Prevention of ventilator-associated pneumonia in the intensive care unit: a review of the clinically relevant recent advancements. The Indian journal of medical research. 2014;139(6):814-21.
31. Mwita JC, Souda S, Magafu M, Massele A, Godman B, Mwandri M. Prophylactic antibiotics to prevent surgical site infections in Botswana: findings and implications. Hospital practice. 2018;46(3):97-102.
32. van der Sandt N, Schellack N, Mabope LA, Mawela MPB, Kruger D, Godman B. Surgical Antimicrobial Prophylaxis Among Pediatric Patients in South Africa Comparing Two Healthcare Settings. The Pediatric infectious disease journal. 2019;38(2):122-6.
33. Mogyoródi B, Dunai E, Gál J, Iványi Z. Ventilator-associated pneumonia and the importance of education of ICU nurses on prevention - Preliminary results. Interv Med Appl Sci. 2016;8(4):147-51.
34. Zarb P, Coignard B, Griskeviciene J, Muller A, Vankerckhoven V, Weist K, et al. The European Centre for Disease Prevention and Control (ECDC) pilot point prevalence survey of healthcare-associated infections and antimicrobial use. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2012;17(46).
35. Ahmed I, Rabbi Md. B, Sultana S. Antibiotic resistance in Bangladesh: A systematic review. International Journal of Infectious Diseases 2019; 80: 54–61.
36. Joshi J. Antibiotic Use and Resistance in Bangladesh: Situation Analysis and Recommendations. 2018. Available at URL: https://cddep.org/publications/bangladesh-situation-analysis-amr/.
37. Mia MF, Mia MAA, Saha D, Khan AA, Chakraborty A. Antibiotic Resistance Pattern and Stewardship Programme in Critical Care Settings. Bangladesh Med Res Counc. Bull 2017; 43: 131-7.
38. Rahman MS, Huda S. Antimicrobial resistance and related issues: An overview of Bangladesh situation. Bangladesh J Pharmacol. 2014; 9: 218-24.
39. Kalanuria AA, Ziai W, Mirski M. Ventilator-associated pneumonia in the ICU. Critical care. 2014;18(2):208.
40. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med. 2005;171(4):388-416.
41. Hunter JD. Ventilator associated pneumonia. Postgrad Med J. 2006;82(965):172-8.
42. Safdar N, Dezfulian C, Collard HR, Saint S. Clinical and economic consequences of ventilator-associated pneumonia: a systematic review. Critical care medicine. 2005;33(10):2184-93.
43. Kollef MH, Hamilton CW, Ernst FR. Economic Impact of Ventilator-Associated Pneumonia in a Large Matched Cohort. Infection Control & Hospital Epidemiology. 2012;33(3):250–6.

44. Wagh H, Acharya D. Ventilator Associated Pneumonia – an Overview. BJMP 2009:2(2) 16-19.
45. Mathai AS, Phillips A, Isaac R. Ventilator-associated pneumonia: A persistent healthcare problem in Indian Intensive Care Units! Lung India. 2016;33(5):512-6.
46. Feng DY, Zhou YQ, Zhou M, Zou XL, Wang YH, Zhang TT. Risk Factors for Mortality Due to Ventilator-Associated Pneumonia in a Chinese Hospital: A Retrospective Study. Medical science monitor. 2019;25:7660-5.
47. Richards M, Thursky K, Buising K. Epidemiology, prevalence, and sites of infections in intensive care units. Seminars in respiratory and critical care medicine. 2003;24(1):3-22.
48. Spencer RC. Epidemiology of infection in ICUs. Intensive care medicine. 1994;20 Suppl 4:S2-6.
49. Wang L, Zhou K-H, Chen W, Yu Y, Feng S-F. Epidemiology and risk factors for nosocomial infection in the respiratory intensive care unit of a teaching hospital in China: A prospective surveillance during 2013 and 2015. BMC infectious diseases. 2019;19(1):145-.
50. Celis R, Torres A, Gatell JM, Almela M, Rodriguez-Roisin R, Agusti-Vidal A. Nosocomial pneumonia. A multivariate analysis of risk and prognosis. Chest. 1988;93(2):318-24.
51. Chevret S, Hemmer M, Carlet J, Langer M. Incidence and risk factors of pneumonia acquired in intensive care units. Results from a multicenter prospective study on 996 patients. European Cooperative Group on Nosocomial Pneumonia. Intensive care medicine. 1993;19(5):256-64.
52. Vincent JL, Bihari DJ, Suter PM, Bruining HA, White J, Nicolas-Chanoin MH, et al. The prevalence of nosocomial infection in intensive care units in Europe. Results of the European Prevalence of Infection in Intensive Care (EPIC) Study. EPIC International Advisory Committee. Jama. 1995;274(8):639-44.
53. Chastre J, Fagon JY. Ventilator-associated pneumonia. Am J Respir Crit Care Med. 2002;165(7):867-903.
54. Elliott RM, Burrell AR, Harrigan PW, Murgo M, Rolls KD, Sibbritt DW, et al. Antimicrobial prescription patterns and ventilator associated pneumonia: findings from a 10-site prospective audit. BMC research notes. 2018;11(1):769.
55. Timsit JF, Esaied W, Neuville M, Bouadma L, Mourvllier B. Update on ventilator-associated pneumonia. F1000Research. 2017;6:2061.
56. Baselski V, Klutts JS, Baselski V, Klutts JS. Quantitative cultures of bronchoscopically obtained specimens should be performed for optimal management of ventilator-associated pneumonia. J Clin Microbiol. 2013;51(3):740-4.
57. Guillamet MCV, Burnham JP, Kollef MH. Novel Approaches to Hasten Detection of Pathogens and Antimicrobial Resistance in the Intensive Care Unit. Seminars in respiratory and critical care medicine. 2019;40(4):454-64.
58. Timsit JF, Bassetti M, Cremer O, Daikos G, de Waele J, Kallil A, et al. Rationalizing antimicrobial therapy in the ICU: a narrative review. Intensive care medicine. 2019;45(2):172-89.
59. Kollef MH, Fraser VJ. Antibiotic resistance in the intensive care unit. Annals of internal medicine. 2001;134(4):298-314.
60. Scholte JB, van Dessel HA, Linssen CF, Bergmans DC, Savelkoul PH, Roekaerts PM, et al. Endotracheal aspirate and bronchoalveolar lavage fluid analysis: interchangeable diagnostic modalities in suspected ventilator-associated pneumonia? J Clin Microbiol. 2014;52(10):3597-604.

61. Ioanas M, Ferrer R, Angrill J, Ferrer M, Torres A. Microbial investigation in ventilator-associated pneumonia. Eur Respir J. 2001;17(4):791-801.
62. Khilnani GC, Arafath TK, Hadda V, Kapil A, Sood S, Sharma SK. Comparison of bronchoscopic and non-bronchoscopic techniques for diagnosis of ventilator associated pneumonia. Indian journal of critical care medicine. 2011;15(1):16-23.
63. Nair S, Sen N, Peter JV, Raj JP, Brahmadathan KN. Role of quantitative endotracheal aspirate and cultures as a surveillance and diagnostic tool for ventilator associated pneumonia: a pilot study. Indian journal of medical sciences. 2008;62(8):304-13.
64. Bergmans DC, Bonten MJ, De Leeuw PW, Stobberingh EE. Reproducibility of quantitative cultures of endotracheal aspirates from mechanically ventilated patients. J Clin Microbiol. 1997;35(3):796-8.
65. Koenig SM, Truwit JD. Ventilator-associated pneumonia: diagnosis, treatment, and prevention. Clinical microbiology reviews. 2006;19(4):637-57.
66. Mathur P, Singh S. Multidrug resistance in bacteria: a serious patient safety challenge for India. Journal of laboratory physicians. 2013;5(1):5-10.
67. Parajuli NP, Acharya SP, Mishra SK, Parajuli K, Rijal BP, Pokhrel BM. High burden of antimicrobial resistance among gram negative bacteria causing healthcare associated infections in a critical care unit of Nepal. Antimicrobial resistance and infection control. 2017;6:67-.
68. Rijal BP, Satyal D, Parajuli NP. High Burden of Antimicrobial Resistance among Bacteria Causing Pyogenic Wound Infections at a Tertiary Care Hospital in Kathmandu, Nepal. Journal of pathogens. 2017;2017:9458218.
69. Zhou Y, Zhang D, Chen Y, Zhou S, Pan S, Huang Y, et al. Healthcare-associated infections and Shanghai clinicians: a multicenter cross-sectional study. PloS one. 2014;9(8):e105838.
70. Fourie T, Schellack N, Bronkhorst E, Coetzee J, Godman B. Antibiotic prescribing practices in the presence of extended-spectrum β-lactamase (ESBL) positive organisms in an adult intensive care unit in South Africa – A pilot study. Alexandria Journal of Medicine. 2018; 54: 541-7
71. Alrubaiee G, Baharom A, Shahar HK, Daud SM, Basaleem HO. Knowledge and practices of nurses regarding nosocomial infection control measures in private hospitals in Sana'a City, Yemen. Safety in Health. 2017;3(1):16.
72. Abdulraheem I, Amodu M, Saka M, Bolarinwa O, Uthman M. Knowledge, awareness and compliance with standard precautions among health workers in north eastearn Nigeria. J Community Med Health Edu. 2012;2(3):1–5.
73. Isara AR, Ofili AN. Knowledge and practice of standard precautions among health care workers in the Federal Medical Centre, Asaba, Delta State, Nigeria. The Nigerian postgraduate medical journal. 2010;17(3):204-9.
74. Lien LTQ, Johansson E, Lan PT, Chuc NTK, Thoa NTM, Hoa NQ, et al. A Potential Way to Decrease the Know-Do Gap in Hospital Infection Control in Vietnam: "Providing Specific Figures on Healthcare-Associated Infections to the Hospital Staff Can 'Wake Them Up' to Change Their Behaviour". International journal of environmental research and public health. 2018;15(7):1549.
75. Nusrat T, Akter N, Haque M, Rahman NAA, Dewanjee AK, Ahmed S, et al. Comparative Study of CDST & Multiplex PCR to Detect MBL Producing Gram-Negative Bacilli among VAP Patients Admitted in a Public Medical College Hospital of Bangladesh. Pathogens. 2019;8(3).

76. Logan LK, Weinstein RA. The Epidemiology of Carbapenem-Resistant Enterobacteriaceae: The Impact and Evolution of a Global Menace. The Journal of infectious diseases. 2017;215(suppl_1):S28-s36.
77. Oteo J, Miro E, Perez-Vazquez M, Navarro F. Evolution of carbapenemase-producing Enterobacteriaceae at the global and national level: what should be expected in the future? Enfermedades infecciosas y microbiologia clinica. 2014;32 Suppl 4:17-23.
78. Goodman KE, Simner PJ, Tamma PD, Milstone AM. Infection control implications of heterogeneous resistance mechanisms in carbapenem-resistant Enterobacteriaceae (CRE). Expert review of anti-infective therapy. 2016;14(1):95-108.
79. Potter RF, D'Souza AW, Dantas G. The rapid spread of carbapenem-resistant Enterobacteriaceae. Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy. 2016;29:30-46.
80. Picão RC, Andrade SS, Nicoletti AG, Campana EH, Moraes GC, Mendes RE, et al. Metallo-beta-lactamase detection: comparative evaluation of double-disk synergy versus combined disk tests for IMP-, GIM-, SIM-, SPM-, or VIM-producing isolates. Journal of clinical microbiology. 2008;46(6):2028-37.
81. Hong DJ, Bae IK, Jang I-H, Jeong SH, Kang H-K, Lee K. Epidemiology and Characteristics of Metallo-β-Lactamase-Producing Pseudomonas aeruginosa. Infect Chemother. 2015;47(2):81-97.
82. Behera B, Mathur P, Das A, Kapil A, Sharma V. An evaluation of four different phenotypic techniques for detection of metallo-beta-lactamase producing Pseudomonas aeruginosa. Indian J Med Microbiol. 2008;26(3):233-7.
83. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-beta-lactamases: the quiet before the storm? Clinical microbiology reviews. 2005;18(2):306-25.
84. Babu KV, Visweswaraiah DS, Kumar A. The influence of Imipenem resistant metallo-beta-lactamase positive and negative Pseudomonas aeruginosa nosocomial infections on mortality and morbidity. Journal of natural science, biology, and medicine. 2014;5(2):345-51.
85. Naylor NR, Atun R, Zhu N, Kulasabanathan K, Silva S, Chatterjee A, et al. Estimating the burden of antimicrobial resistance: a systematic literature review. Antimicrob Resist Infect Control. 2018;7:58.
86. Kaur A, Gupta V, Chhina D. Prevalence of metalo- β-lactamase-producing (MBL) Acinetobacter species in a tertiary care hospital. Iranian journal of microbiology. 2014;6(1):22-5.
87. Hodiwala A, Dhoke R, Urhekar AD. Incidence of metallo-beta-lactamase producing Pseudomonas, Acinetobacter and enterobacterial isolates in hospitalised patient. Int J Pharm Bio Sci. 2013; 3: 79–83.
88. Ghasemian A, Salimian Rizi K, Rajabi Vardanjani H, Nojoomi F. Prevalence of Clinically Isolated Metallo-beta-lactamase-producing Pseudomonas aeruginosa, Coding Genes, and Possible Risk Factors in Iran. Iran J Pathol. 2018;13(1):1-9.
89. Jaurin B, Grundström T. ampC cephalosporinase of Escherichia coli K-12 has a different evolutionary origin from that of beta-lactamases of the penicillinase type. Proceedings of the National Academy of Sciences of the United States of America. 1981;78(8):4897-901.

90. Ouellette M, Bissonnette L, Roy PH. Precise insertion of antibiotic resistance determinants into Tn21-like transposons: nucleotide sequence of the OXA-1 beta-lactamase gene. Proceedings of the National Academy of Sciences of the United States of America. 1987;84(21):7378-82.
91. Rasmussen BA, Bush K. Carbapenem-hydrolyzing beta-lactamases. Antimicrobial agents and chemotherapy. 1997;41(2):223-32.
92. Deshmukh DG, Damle AS, Bajaj JK, Bhakre JB, Patwardhan NS. Metallo-β-lactamase-producing clinical isolates from patients of a tertiary care hospital. Journal of laboratory physicians. 2011;3(2):93-7.
93. Khaledi M, Shahini Shams Abadi M, Validi M, Zamanzad B, Vafapour R, Gholipour A. Phenotypic and genotypic detection of metallo-β-lactamases in A. baumanii isolates obtained from clinical samples in Shahrekord, southwest Iran. BMC research notes. 2019;12(1):597-.
94. Emeraud C, Escaut L, Boucly A, Fortineau N, Bonnin RA, Naas T, et al. Aztreonam plus Clavulanate, Tazobactam, or Avibactam for Treatment of Infections Caused by Metallo-β-Lactamase-Producing Gram-Negative Bacteria. Antimicrobial agents and chemotherapy. 2019;63(5):e00010-19.
95. Craven DE, Chroneou A, Zias N, Hjalmarson KI. Ventilator-associated tracheobronchitis: the impact of targeted antibiotic therapy on patient outcomes. Chest. 2009;135(2):521-8.
96. Richards MJ, Edwards JR, Culver DH, Gaynes RP. Nosocomial infections in combined medical-surgical intensive care units in the United States. Infection control and hospital epidemiology. 2000;21(8):510-5.
97. Jones RN. Microbial etiologies of hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2010;51 Suppl 1:S81-7.
98. Alcon A, Fabregas N, Torres A. Hospital-acquired pneumonia: etiologic considerations. Infectious disease clinics of North America. 2003;17(4):679-95.
99. Kollef MH, Shorr A, Tabak YP, Gupta V, Liu LZ, Johannes RS. Epidemiology and outcomes of health-care-associated pneumonia: results from a large US database of culture-positive pneumonia. Chest. 2005;128(6):3854-62.
100. Guckan R, Kilinc C, Demir AD, Capraz A, Yanik K. Antimicrobial Susceptibility of Acinetobacter baumannii complex Isolated from Different Clinical Samples in A Tertiary Care Hospital. J Antibiot Res. 2015; 1(1): 103.
101. Fatema K, Ahsan ASM, Barai L, Ahmed F, Has JA, Faruq MO. Bacterial profile and their antibiotic resistance in an ICU of Bangladesh: Comparison of four studies from 2004 to 2011. Bangladesh Crit Care J September 2016; 4 (2): 79-85.
102. Sarkar MD, Raj HJ, Ghosh T. Ventilator-Associated Pneumonia, a challenge in intensive care unit acquired infection. Bang J Med Sci. 2016; 15(4): 588-595.
103. Tewari R, Chopra D, Wazahat R, Dhingra S, Dudeja M. Antimicrobial Susceptibility Patterns of an Emerging Multidrug Resistant Nosocomial Pathogen: Acinetobacter baumannii. The Malaysian journal of medical sciences. 2018;25(3):129-34.
104. Farzana R, Jones LS, Rahman MA, Toleman MA, Sands K, Portal E, et al. Emergence of mcr-1 mediated colistin resistant Escherichia coli from a hospitalized patient in Bangladesh. Journal of infection in developing countries. 2019;13(8):773-6.

105. Islam A, Rahman Z, Monira S, Rahman MA, Camilli A, George CM, et al. Colistin resistant Escherichia coli carrying mcr-1 in urban sludge samples: Dhaka, Bangladesh. Gut Pathog. 2017;9:77-.
106. Mendelson M, Brink A, Gouws J, Mbelle N, Naidoo V, Pople T, et al. The One Health stewardship of colistin as an antibiotic of last resort for human health in South Africa. The Lancet Infectious diseases. 2018;18(9):e288-e94.
107. Babakhani S, Derikvand SS, Nazer MR, Kazemi MJ. Comparison frequency and Determination of antibiotic resistance pattern of Klebsiella SPP. isolated from Nosocomial infection in Khorramabad Shohadaye Ashayer hospital. Bull Env Pharmacol Life Sci. 2014; 3 (12): 149-154. .
108. Dominic RML, Prashant, Shiny S, Baliga S. A clinico-microbiological study of ventilator-associated pneumonia in a tertiary care hospital. Int J Biol Med Res.2012; 3(2):1651-1654.
109. Chang L, Dong Y, Zhou P. Investigation on Risk Factors of Ventilator-Associated Pneumonia in Acute Cerebral Hemorrhage Patients in Intensive Care Unit. Canadian respiratory journal. 2017;2017:7272080.
110. Ding C, Zhang Y, Yang Z, Wang J, Jin A, Wang W, et al. Incidence, temporal trend and factors associated with ventilator-associated pneumonia in mainland China: a systematic review and meta-analysis. BMC Infect Dis. 2017;17(1):468.
111. Liu Y, Di Y, Fu S. Risk factors for ventilator-associated pneumonia among patients undergoing major oncological surgery for head and neck cancer. Frontiers of medicine. 2017;11(2):239-46.
112. Sharpe JP, Magnotti LJ, Weinberg JA, Brocker JA, Schroeppel TJ, Zarzaur BL, et al. Gender disparity in ventilator-associated pneumonia following trauma: identifying risk factors for mortality. The journal of trauma and acute care surgery. 2014;77(1):161-5.
113. Cui JB, Chen QQ, Liu TT, Li SJ. Risk factors for early-onset ventilator-associated pneumonia in aneurysmal subarachnoid hemorrhage patients. Brazilian journal of medical and biological research. 2018;51(7):e6830.
114. Nathwani D, Varghese D, Stephens J, Ansari W, Martin S, Charbonneau C. Value of hospital antimicrobial stewardship programs [ASPs]: a systematic review. Antimicrob Resist Infect Control. 2019;8:35.
115. Fadare JO, Ogunleye O, Iliyasu G, Adeoti A, Schellack N, Engler D, et al. Status of antimicrobial stewardship programmes in Nigerian tertiary healthcare facilities: Findings and implications. Journal of global antimicrobial resistance. 2018;17:132-6.
116. Kalungia AC, Mwambula H, Munkombwe D, Marshall S, Schellack N, May C, et al. Antimicrobial stewardship knowledge and perception among physicians and pharmacists at leading tertiary teaching hospitals in Zambia: implications for future policy and practice. Journal of chemotherapy. 2019: 31 (7-8): 378-387
117. Anand Paramadhas BD, Tiroyakgosi C, Mpinda-Joseph P, Morokotso M, Matome M, Sinkala F, et al. Point prevalence study of antimicrobial use among hospitals across Botswana; findings and implications. Expert review of anti-infective therapy. 2019;17(7):535-46.
118. Charani E, Smith I, Skodvin B, Perozziello A, Lucet JC, Lescure FX, et al. Investigating the cultural and contextual determinants of antimicrobial stewardship programmes across low-, middle- and high-income countries-A qualitative study. PloS one. 2019;14(1):e0209847.

119. Cox JA, Vlieghe E, Mendelson M, Wertheim H, Ndegwa L, Villegas MV, et al. Antibiotic stewardship in low- and middle-income countries: the same but different? Clinical microbiology and infection. 2017;23(11):812-8.
120. Alowi M, Kani Y. Impact of Pharmaceutical Companies' Promotional Tools on Physicians' Prescription Patterns: A Systematic Review. J Appl Pharm 2018; 10: 267.
121. Mohiuddin M, Rashid SF, Shuvro MI, Nahar N, Ahmed SM. Qualitative insights into promotion of pharmaceutical products in Bangladesh: how ethical are the practices? BMC medical ethics. 2015;16(1):80.
122. Islam S, Rahman A, Al-Mahmood AK. Bangladesh Pharmaceutical Industry: Perspective and the Prospects. Bangladesh Journal of Medical Science 2018; 17 (4): 519-525.
123. Sharmin R, Sharmin ZR, Md. Mosaddek AS, Islam MZ, Rahman MF, Parvin R, et al. Medicine promotional literature as a source of updated information in Bangladesh: Do those advertising literature promote continued medical education or deceptive advertising? Acta Med Int 2017;4:20-5.
124. Fadare JO, Oshikoya KA, Ogunleye OO, Desalu OO, Ferrario A, Enwere OO, et al. Drug promotional activities in Nigeria: impact on the prescribing patterns and practices of medical practitioners and the implications. Hospital practice. 2018;46(2):77-87.
125. Ogunleye OO, Fadare JO, Yinka-Ogunleye AF, Anand Paramadhas BD, Godman B. Determinants of antibiotic prescribing among doctors in a Nigerian urban tertiary hospital. Hospital practice. 2019;47(1):53-8.
126. Fadare JO, Ogunleye O, Obiako R, Orubu S, Enwere O, Ajemigbitse AA, et al. Drug and therapeutics committees in Nigeria: evaluation of scope and functionality. Expert review of clinical pharmacology. 2018;11(12):1255-62.
127. Matlala M, Gous AG, Godman B, Meyer JC. Structure and activities of pharmacy and therapeutics committees among public hospitals in South Africa; findings and implications. Expert review of clinical pharmacology. 2017;10(11):1273-80.
128. Mashaba TP, Matlala M, Godman B, Meyer JC. Implementation and monitoring of decisions by pharmacy and therapeutics committees in South African public sector hospitals. Expert review of clinical pharmacology. 2019;12(2):159-68.
129. Lima-Dellamora Eda C, Caetano R, Gustafsson LL, Godman BB, Patterson K, Osorio-de-Castro CG. An analytical framework for assessing drug and therapeutics committee structure and work processes in tertiary Brazilian hospitals. Basic & clinical pharmacology & toxicology. 2014;115(3):268-76.
130. Nakwatumbah S, Kibuule D, Godman B, Haakuria V, Kalemeera F, Baker A, et al. Compliance to guidelines for the prescribing of antibiotics in acute infections at Namibia's national referral hospital: a pilot study and the implications. Expert review of anti-infective therapy. 2017;15(7):713-21.
131. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clinical infectious diseases 2009;49(11):1749-55.