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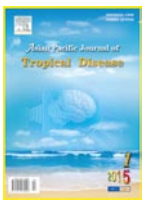

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The bad, the ugly and the demon: a tale of extensively drug-resistant, extended-spectrum-beta-lactamase- and metallo-beta-lactamase-producing superbugs associated with nosocomial pneumonia

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PEER REVIEW

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Comments

The work reports on issue of nosocomial infection with specific use of investigation to support the hypothesis. The work is interesting and can be applicable in tropical medicine. Also, it is useful for clinical pharmacology aspect. The work can be further referenced in the field.
Details on Page 75

ABSTRACT

Objective: To determine the bacterial etiology of nosocomial pneumonia (NP) and to assess the current levels of antimicrobial resistance with special reference to the status of extended-spectrum-beta-lactamase (ESBL) and metallo-beta-lactamase (MBL)-producing bacterial strains in a university hospital of Nepal.

Methods: A total of 60 specimens (sputum and endotracheal secretion) from patients diagnosed of NP were collected and processed following standard methodology. Combined disk and double disk synergy test method were used for the detection of ESBL. Ethylene-diamine-tetraacetic acid-based combined disk method was used for the detection of MBL-producing isolates.

Results: Out of total 60 specimens, 85% yielded significant mixed bacterial growth. *Acinetobacter* spp. was the most predominant isolate (30.43%) followed by *Klebsiella* spp. (28.98%), *Pseudomonas aeruginosa* (17.39%), *Escherichia coli* and *Staphylococcus aureus* (*S. aureus*) (8.69% for each). All *Escherichia coli*, *Klebsiella* spp. and *S. aureus* were multidrug resistant. Nearly 76% of *Acinetobacter* spp. were extensively drug resistant. MBL was seen in 25.3% of the Gram-negative isolates. *Acinetobacter* spp. was the most frequent MBL-producer (15.9%). ESBL was present in 41.3% of Gram-negative isolates. Tigecycline and polymyxin B followed by carbapenems, cefoperazone-sulbactam, piperacillin-tazobactam and amikacin were the most effective antibiotics for drug-resistant Gram-negative bacteria. All isolates of *S. aureus* were methicillin-resistant; however, they were susceptible to vancomycin, linezolid, quinupristin-dalfopristin and tigecycline.

Conclusions: High prevalence of drug resistance among the isolates of NP has demanded cautious selection of antibiotics. Further studies should be done in our setting to find out genes responsible for drug resistance. Last but not least, we advocate for the development of new antibiotics.

KEYWORDS

Nosocomial pneumonia, Extended-spectrum-beta-lactamase, Metallo-beta-lactamase, extensively drug resistant

1. Introduction

Nosocomial pneumonia (NP) is an infection of the lung parenchyma that is neither present nor incubating at the time of hospital admission and which develops after 48 hours of hospital admission. It is the second most frequent nosocomial infection but the first in terms of morbidity, mortality and cost[1]. In the intensive care units (ICU), it is the most frequent nosocomial infection because of the severity of underlying diseases, the frequency of invasive interventions, and the frequent use of broad-spectrum antibiotics[2].

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The nosocomial infections contribute to the emergence of resistant strains like multidrug resistant (MDR), methicillin-resistant *Staphylococcus aureus* (*S. aureus*) (MRSA), extended-spectrum-beta-lactamase (ESBL)- and metallo-beta-lactamase (MBL)-producing organisms due to antibiotic selection pressure. As the pathogens causing hospital-based pneumonia become more drug resistant, clinical trial designs become more complex, thus making monotherapeutic protocols nearly impossible and the analyses of trial results extremely difficult[3]. Recently, a high level of antibiotic resistance in lower respiratory tract pathogens, exacerbated by the association of ESBL and MBL, has been seen in Nepal[4]. These strains may be extensively drug resistant (XDR). The emergence of such strains in nosocomial pneumonia drastically compromise effective treatments, bringing us closer to the much feared 'end of antibiotics'.

The incidence of MDR pathogens is not decreasing, despite the attempts of antibacterial stewardship and rigorous endeavor to infection control of MDR bacteria in hospital. Besides, these bad bugs may escape the hospital and join the ranks of the community pathogens[5]. This is a worrying public health issue as infections caused by such enzyme-producing organisms are associated with a higher morbidity and mortality, and greater economic burden to developing countries like Nepal as these enzymes can be carried on bacterial chromosomes, that is, inherent to the organism, or may be plasmid-mediated with the potential to move between bacterial populations.

2. Materials and methods

A prospective study was done among the inpatients of Tribhuvan University Teaching Hospital diagnosed with nosocomial pneumonia from May to August, 2012. Endotracheal secretion or sputum sample, as received in the laboratory from patients meeting criteria for NP as defined by Center for Disease Control, was processed following standard methodology[6].

2.1. Antimicrobial sensitivity testing

Antibiotic susceptibility test of all the isolates was done by using Mueller Hinton agar (MHA) (Oxoid, UK) by the standard disk diffusion technique of Kirby-Bauer method and interpreted as per Clinical and Laboratory Standards Institute (CLSI) recommendations[7].

2.2. MRSA screening

MRSA screening was done using cefoxitin disk (30 μg) method as recommended by CLSI. Organisms were deemed methicillin resistant when zone of inhibition (ZOI) was ≤21 mm for *S. aureus*[7].

2.3. Tests for ESBL-production in Gram-negative isolates

2.3.1. ESBL screening test

According to CLSI guidelines, strains showing ZOI of ≤22 mm for ceftazidime (CAZ) (30 μg), ≤27 mm for cefotaxime (CTX) (30 μg), and ≤25 mm for ceftriaxone (CRO) (30 μg) were considered potential ESBL- producer and were selected for confirmational tests of ESBL[7].

2.3.2. ESBL confirmatory tests

2.3.2.1. Combination disk method

CAZ (30 μg) and CTX (30 μg) disks alone and in combination with clavulanic acid (10 μg) were placed 25 mm apart. An increase of ≥5 mm in ZOI for ceftazidime-clavulanic acid (30/10 μg) and cefotaxime-clavulanic acid (30/10 μg) compared to CAZ and CTX alone was confirmed as ESBL producers[7].

2.3.2.2. Double disc synergy test

Three discs including CAZ (30 μg), CTX (30 μg), and CRO (30 μg) were placed around the centrally placed disc of amoxycillin-clavulanic acid (amoxyclav) (20/10 μg) with an edge to edge distance of 15 mm. The isolates showing enhancement of the ZOI and synergy to centrally placed disk of amoxyclav (20/10 μg) for one or more of the discs after overnight incubation at 37°C was considered as the ESBL producer[4].

2.4. MBL screening test

The isolates were subjected for MBL detection when the ZOI for CAZ (30 μg) was <18 mm[8].

2.4.1. MBL confirmation by combination disk method

Two imipenem (IPM) disks (10 μg) were used. In one of them, 10 μL of 0.1 mol/L (292 μg) anhydrous ethylenediamine-tetraacetic acid (EDTA) was added. Then the two disks were placed 25 mm apart (center to center). An increase in zone diameter of > 4 mm around the IPM-EDTA disk compared to that of the IPM disk alone was considered positive for an MBL[7].

2.5. Definition of MDR and XDR

MDR *Acinetobacter* spp. were defined as the isolates of *Acinetobacter* spp. resistant to at least three classes of antimicrobial agents-all penicillins and cephalosporins

(including inhibitor combinations), fluoroquinolones, and aminoglycosides[9].

XDR *Acinetobacter* spp. were defined as the isolates of *Acinetobacter* spp. that were resistant to the three classes of antimicrobials described above (MDR) along with carbapenems[9].

MDR among *Pseudomonas aeruginosa* (*P. aeruginosa*) was defined as resistance to at least 3 of the following antimicrobial groups: cephalosporins (ceftazidime or cefepime), aminoglycosides, fluoroquinolones, carbapenems, and antipseudomonal penicillins (piperacillins)[10].

MDR among *Klebsiella* species, and *Escherichia coli* (*E. coli*) was defined as resistance to at least 3 of the following antimicrobial groups: third- or fourth-generation cephalosporins, aminoglycosides, fluoroquinolones, piperacillins, and ampicillin-sulbactam[10].

Isolates of *S. aureus*, *Citrobacter fruendii* and *Burkholdenia cepacia* (*B. cepacia*) complex were labelled as MDR if they were non-susceptible to at least one agent in three or more classes of antimicrobial agents[11].

2.6. D-zone test for inducible macrolide-lincosamide-streptogramins B (iMLSB)

In *S. aureus*, iMLSB resistance was detected by disk approximation test placing a 2 μg clindamycin disk 15 mm away from the edge of a 15 μg erythromycin disk on a Mueller-Hinton agar plate. Following incubation, organisms that showed "D" zone of the clindamycin disk adjacent to the erythromycin disk were considered to exhibit inducible clindamycin resistance[7].

2.7. Data processing and analysis

Data were analyzed using Microsoft Excel 2007 and represented as frequency distribution and percentage.

2.8. Ethical consideration

Ethical approval was obtained from the Institutional Review Board at the Institute of Medicine, Tribhuvan University, Kathmandu.

3. Results

A total of 60 samples including sputum (n=44) and endotracheal secretion (n=16) were taken from patients suffering from NP. Samples were processed in the bacteriology laboratory as per American Society for Microbiology Guidelines.

3.1. Distribution of different bacterial isolates

Out of total 60 specimens, there was mixed bacterial growth in 9 specimens (15%) and monomicrobial growth in 51 specimens (85%). Out of total bacterial isolates (n=69), 91.3% (n=63) were Gram-negative and 8.7% (n=6) were Gram-positive.

Among these 69 bacterial isolates, *Acinetobacter* spp. was the most common one (30.43%) (n=21), followed by *K. pneumoniae* (28.98%) (n=20) and *P. aeruginosa* (17.39%) (n=12) (Figure 1).

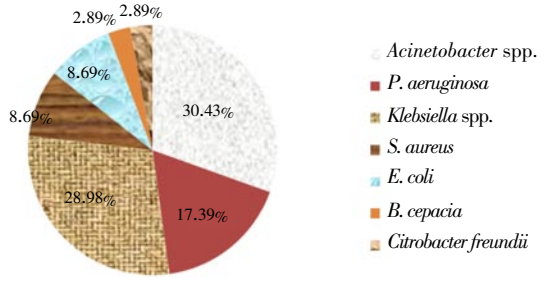

Figure 1. Distribution of different bacterial isolates (n=69).

3.2. Antibiogram of Acinetobacter spp.

All the isolates (n=21) were found to be sensitive to polymyxin B and tigecycline. However, all were resistant to cefotaxime (Table 1).

Table 1
Antibiogram of *Acinetobacter* spp. (n=21).

Antibiotic	Sensitive		Resistance	
	No.	%	No.	%
Ciprofloxacillin	4	19.0	17	81.0
Levofloxacin	5	23.8	16	76.2
Cotrimoxazole	2	9.5	19	91.0
Cefotaxime	0	0.0	21	100.0
Cefoperazone-sulbactam	5	23.8	16	76.2
Piperacillin-tazobactam	5	23.8	16	76.2
Imipenem	5	23.8	16	76.2
Cefipime	3	14.2	18	85.8
Amikacin	6	28.5	15	71.5
Polymyxin B	21	100.0	0	0.0
Doxycycline	10	47.6	12	52.4
Chloramphenicol	2	9.5	19	90.5
Tigecycline	21	100.0	0	0.0

Among the 21 *Acinetobacter* isolates, 95.25% (n=20) were MDR, 76.19% (n=16) were XDR, 47.60% (n=10) were MBL producer, 14.20% (n=3) were ESBL producer while none were MBL and ESBL co-producer.

3.3. Antibiotics sensitivity of Gram-negative isolates

Tigecycline and polymyxin B, followed by imipenem, meropenem, cefoperazone-sulbactam, piperacillin-

tazobacam and amikacin were the most effective antibiotics for most of the Gram-negative isolates (Table 2).

Table 2
Antibiotics sensitivity of Gram-negative isolates.

Antibiotics used	Sensitive (%)	Resistance (%)
Polymyxin B*	100.00	0.00
Tigecycline**	100.00	0.00
Imipenem	79.24	20.75
Cefoperazone-sulbactam	71.69	28.30
Piperacillin-tazobactam	67.92	32.07
Amikacin	45.20	54.71
Chloramphenicol	39.60	60.40
Levofloxacin	30.20	69.80
Cefipime	26.40	73.80
Ciprofloxaciin	20.80	79.20

*: Except *B. cepacia* complex; **: Except *P. aeruginosa*.

3.4. Distribution of nosocomial isolates in different wards

Table 3 shows that incidence of nosocomial pneumonia was more common in ICU (49.3%). *Acinetobacter* spp. was the most common isolate causing nosocomial pneumonia in ICU (41.2%, *n*=14).

3.5. Pattern of MDR, ESBL, MBL, MRSA and iMLSB among different wards

As shown in Table 4, MDR, ESBL, MBL and MRSA in nosocomial isolates were more common in ICU. All isolates of *S. aureus* were methicillin resistant (*n*=6). However, all of them were susceptible to vancomycin, linezolid, quinupristin-dalfopristin and tigecycline.

Table 3
Distribution of nosocomial isolates among different wards (*n*=69).

Wards	*Acinetobacter* spp.	*K. pneumoniae*	*P. aeruginosa*	*E. coli*	*S. aureus*	*B. cepacia* complex	*Citrobacter* spp.	Total
ICU	14	6	6	2	3	2	1	34
Neuro	3	1	4	0	0	0	0	8
Burn	1	0	0	0	0	0	0	1
Medical	2	10	1	3	2	0	0	18
Surgical	1	3	1	1	1	0	1	8
Total	21	20	12	6	6	2	2	69

Table 4
Pattern of MDR, ESBL, MBL, MRSA and iMLSB in different wards.

Wards	ESBL	MBL	MRSA	iMLSB	MDR
ICU	11	9	3	0	29
Neuro	2	2	0	0	6
Burn	0	1	0	0	1
Medical	10	2	2	1	16
Surgical	3	2	1	0	8
Total	26	16	6	1	60

3.6. Pattern of MDR, ESBL and MBL among Gram negative isolates (n=63)

MBL production was seen in maximum number among the isolates of *Acinetobacter* spp. *K. pneumoniae* was the most frequent isolate to produce ESBL. Equal number of *Acinetobacter* spp. and *K. pneumoniae* were found to be MDR (*n*=20) (Table 5).

Table 5
Pattern of MDR, ESBL and MBL among Gram negative isolates (*n*=63)

Organisms	No. of cases	Total %	MBL No.	MBL %	ESBL No.	ESBL %	MDR No.	MDR %
Acinetobacter spp.	21	33.3	10	15.9	3	4.7	20	31.7
K. pneumoniae	20	31.7	5	7.9	16	25.4	20	31.7
P. aeruginosa	12	19.0	1	1.6	1	1.6	6	9.5
E. coli	6	9.5	0	0.0	6	9.5	6	9.5
B. cepacia complex	2	3.1	0	0.0	0	0.0	1	1.6
Citrobacter spp.	2	3.1	0	0.0	0	0.0	1	1.6

4. Discussion

This study was undertaken with 60 specimens with a view to explore the pathogens associated with NP and to guide the clinicians with the most appropriate antibiotic against those pathogens.

In this study, there was mixed bacterial growth in 9 specimens. Colonization of ventilators often occurs with more than one type of organism which may lead to mixed bacterial infection as seen in other studies[12,13]. Out of total 69 nosocomial isolates, majority were Gram-negative (91.3%). This was similar to other studies in which Gram-negative bacteria were seen as the most common etiological agents of hospital-acquired pneumonia[14]. This is probably due to the increased rate of Gram-negative colonization in oropharyngeal specimens during hospitalization[15].

Acinetobacter spp. appeared to be predominant isolates 30.43% (*n*=21) causing NP which is in accordance to the finding of Singhal *et al*[13]. *Acinetobacter* spp. was followed by *K. pneumoniae* 28.98% (*n*=20), *P. aeruginosa* 17.39% (*n*=12), *E. coli* and *S. aureus* each with 8.69% (*n*=6). *B. cepacia* complex and *Citrobacter freundii* were least frequent isolates.

Out of total 69 bacteria isolated, 86.95% were MDR. Out of 21 *Acinetobacter* isolates, 95.25% (*n*=20) were MDR and 76.19% (*n*=16) were XDR. Isolates of *Acinetobacter* spp. were resistant to most of the antibiotics used. In the last few years, resistance to antibacterial drugs has been increasing in

Acinetobacter spp. which has become a substantial treatment challenge in Nepal[16]. The resistance of *Acinetobacter* spp. towards the carbapenems was high in this study (76.2% each for imipenem and meropenem). In a Turkish study, rates of resistance to carbapenems had been 0% for imipenem and 20% for meropenem in 2009, while these rates raised to very high values with 88.4% for imipenem and 93.7% for meropenem in 2011[17]. This shows that *Acinetobacter* are emerging with resistance against carbapenems, leading to very few therapeutic options. Unfortunately, *Acinetobacter* isolates showed poor susceptibility towards doxycycline (47.6%), amikacin (28.5%), piperacillin-tazobactam and cefoperazone-sulbactam (23.8% each). One of the mechanisms for decreased susceptibility to carbapenems may be because of carbapenemase (*e.g.*, MBL) production[18]. In this study, 47.6% of the *Acinetobacter* isolates were MBL producer. Regarding *Pseudomonas* spp., 50% were MDR, and one isolate was MBL and ESBL co-producer. Likewise, MDR isolates were widely present among Enterobacteriaceae. All *K. pneumoniae* and *E. coli* isolates were MDR. The emergence and increasing trend of MDR among *E. coli* has been reported by others too[3].

For most of the Gram negative isolates, tigecyline and polymyxin B had the widest coverage followed by carbapenems, cefoperazone-sulbactam and piperacillin-tazobactam. Tigecycline showed excellent activity against a bunch of difficult-to-treat pathogens (except *P. aeruginosa*) currently encountered in the hospital setting[19]. But the decreased susceptibility of Gram-negative isolates towards the third generation and fourth generation cephalosporins-cefotaxime, and cefipime (<30%) could be attributed to ESBL or Amp C β-lactamase producers or some other relevant underlying mechanisms. This study showed that 41.3% of the Gram-negative isolates were ESBL producers, which is tremendously higher as compared to 4.6% reported previously in the same setting[12]. This is a terrific condition. Around seventy-seven percent of Enterobacteriaceae and twelve percent of nonfermenters were ESBL-producers. All ESBL producers were MDR. Susceptibility of Gram negative isolates towards aminoglycosides, fluoroquinolones and cotrimoxazole, was less than 50%. It is again alarming to note that all isolates of *S. aureus* were methicillin resistant. One of them was of iMLSB phenotype.

In nosocomial infections, carbapenems are used as the last resort for the treatment of MDR Gram-negative bacterial infection[16]. However, resistance to these "miracle" antimicrobials has been increasingly reported worldwide including Nepal[20]. This resistance is mainly mediated by MBLs. In this study, MBL was present in 25.3% of Gram-negative isolates. This is astonishingly higher as compared to the 2.9% in the same hospital in 2008[20]. Out of total 63 Gram negative bacteria, 47.6% of *Acinetobacter*, 30.0% of *Klebsiella* spp. and 8.3% of *Pseudomonas* isolates were MBL-producer. The MBL-producing *Klebsiella* spp. in the present study was higher in number than that shown by Shrestha *et al.* (4.17%) [12].

ICU patients acquire nosocomial infections at a much higher rate than patients elsewhere in the hospital supporting the present finding of the high occurrence of nosocomial pneumonia in ICU than other wards (49.3%). Multidrug resistant ESBL, MBL and MRSA were more common in ICU. Heavy administration of antibiotics in ICU patients along with selection and persistence of highly resistant strains could account for such a high finding[21].

This study underlines the increasing antibacterial resistance among nosocomial isolates which has created a therapeutic challenge for the clinicians and microbiologists. This reflects need for early detection and prompt installation of infection control measures to prevent further spread of resistant mechanism to other bacteria. Additionally, it is also important to establish and follow antibiotic stewardship in our hospital. In global aspect, to address near "end of antibiotics", newer antibiotics should be developed and there must be judicious use of present antibiotics.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

We are grateful to Research Department of Institute of Medicine, Tribhuvan University for providing in-house faculty grant (Ref: 6-11-E) to conduct this study.

Comments

Background

It is a work that uses laboratory investigation and answers the problem on drug resistance, which can be a good reference data for further research and development in clinical medicine.

Research frontiers

The work brings concern on proper usage of antibiotic. Although this is an old concept, the story in the present case is interesting and can be the new view in this specific issue in medicine. The work can be useful to general reader for further following in routine clinical practice.

Related reports

As noted, there are some previous reports on this aspect.

Since it is an old classical rule in general practice to prevent the drug resistance, it can be expected in any presentation in the present work. However, due to the new view in approach in this report, the work can still be useful for the general reader to use in general practice.

Innovations & breakthroughs

Some new information on the specific issue in clinical pharmacology can be expected. This also leads to the specific consideration in infectious medicine. The work can be further referenced in the field of general medicine.

Applications

This report can be applied in general medicine, pharmacology and infectious medicine. Also, it is a specific issue in nosocomial infection which can be further applied in specific infection control and clinical epidemiology science.

Peer review

The work reports on issue of nosocomial infection with specific use of investigation to support the hypothesis. The work is interesting and can be applicable in tropical medicine. Also, it is useful for clinical pharmacology aspect. The work can be further referenced in the field.

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