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Short Communication

Epidemiology of multidrug-resistant Gram-negative pathogens isolated from ventilator-associated pneumonia in ICU patients

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ABSTRACT

Objectives: Antibiotic resistance among Gram-negative pathogens isolated from ventilator-associated pneumonia (VAP) poses a grave threat in intensive care unit (ICU) patients. The aim of this study was to assess the prevalence of pathogens in ICU patients and their drug resistance profile. The prevalence of extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases and metallo-β-lactamases (MBLs) was also assessed.

Methods: Tracheal aspirates were collected aseptically from 87 ICU patients between May 2012 and January 2014. Cultured isolates were identified by standard microbiological techniques. Antimicrobial susceptibility testing was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines. ESBLs and AmpC β-lactamases were detected both phenotypically and genotypically; MBLs were detected phenotypically.

Results: A total of 77 isolate were cultured. Gram-negative bacteria comprised 68 (88.3%) of the total isolates, among which 49 (72.1%) were multidrug-resistant (MDR). Gram-positive organisms comprised four (5.2%) of the total isolates and all four (100%) were MDR. *Aspergillus fumigatus* (6.4%) was the only fungal pathogen identified.

Conclusions: Pseudomonas aeruginosa was the predominant pathogen associated with VAP. The rising trend of antibiotic resistance in Gram-negative organisms is alarming. Regular monitoring of the pattern of resistance in ICUs is critical in effective management of VAP patients.

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1. Introduction

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

Tracheostomised patients are colonised or infected with endogenous and exogenous bacteria. Risk factors for colonisation or infection with multidrug-resistant (MDR) bacterial species include prolonged length of hospital stay, exposure to an ICU, receipt of mechanical ventilation, colonisation pressure, exposure to broad-spectrum antimicrobial agents, recent surgery, invasive procedures and underlying severity of illness [3]. 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 (ESBLs), plasmid-mediated AmpC β-lactamases (pAmpCs) and metallo-β-lactamases (MBLs) [4]. Infections caused by MDR bacteria expressing β-lactamases pose a serious challenge to clinicians because these bacteria are resistant to a broad range of β-lactams, including third-generation cephalosporins; nosocomial infections caused by these organisms complicate therapy and limit treatment options [5]. The emergence and spread of antimicrobial resistance due to the production of β-lactamases has drawn attention to a need for better diagnostic techniques and newer drugs to allow more specific therapy. Therefore, characterisation and determination of the 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 MDR bacteria. Hence, this study was undertaken to detect the prevalence of and to determine the antimicrobial resistance pattern of clinically relevant bacteria

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producing ESBLs, AmpC β-lactamases and MBLs from tracheal aspirates of patients admitted to the ICU.

2. Materials and methods

This prospective study was carried out on ICU patients of J.N. Medical College (JNMC) of Aligarh Muslim University (Aligarh, Uttar Pradesh, India) from May 2012 to January 2014. JNMC is a tertiary and teaching hospital with 1050 beds and a combined medical and surgical ICU with a 10-bed capacity.

Tracheal aspirates were collected aseptically from 87 ICU patients and a complete history was taken from each patient. The study was performed after approval from the Institutional Ethics Committee of JNMC, and informed written consent was received from the patients. None of the patients or their representatives declined to take part in the study. Patients were chosen consecutively and an endotracheal aspirate was obtained from each patient.

2.1. Specimen collection

Samples were collected in a mucus trapper by applying negative pressure through an automated machine by an experienced physician. Samples were immediately transported to the Department of Microbiology of JNMC.

2.2. Specimen culture

Specimens were inoculated on blood agar, MacConkey agar and chocolate agar plates [6].

2.3. Isolate identification and antibiotic susceptibility testing

Isolates were identified on the basis of standard microbiological techniques [6]. Antibiotic sensitivity testing was performed using the Kirby–Bauer disk diffusion method, and sensitivity results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [7]. Fungal culture was performed on two Sabouraud dextrose agar slants by rolling over the surface and subsequently also in brain–heart infusion (BHI) broth. One tube was incubated at 25 °C and the remaining tube and BHI broth were incubated at 37 °C. Following initial inoculation and incubation, tubes were examined daily for fungal growth for up to 3 weeks. Multidrug resistance was defined as resistance to three or more antimicrobial agents belonging to different structural classes [8].

2.4. Phenotypic tests for extended-spectrum β-lactamase production

Isolates were first screened for the production of ESBL by the disk diffusion method (screening test) using cefotaxime, ceftriaxone, cefepime and ceftazidime [5] and were later confirmed by the cephalosporin/clavulanic acid combination disk (disk potentiation test) [4] and double-disk synergy test [4,5]. *Escherichia coli* ATCC 25922 (non-ESBL-producer) was used as control strain.

2.5. Phenotypic methods for AmpC detection

Cefoxitin disks were used to screen for AmpC-producers by the disk diffusion method [6]. Isolates that were resistant to cefoxitin were considered as potential AmpC-producers.

2.6. Phenotypic methods for metallo-β-lactamase detection

Isolates were tested for sensitivity to imipenem (10 μg) using the Kirby–Bauer method as recommended by the CLSI [7,9]. All isolates with a zone of inhibition of ≤16 mm or that demonstrated heaping, or if the zone was >16 mm but ≤20 mm, were tested for MBL production. There is no CLSI guideline for MBL detection available for *Pseudomonas aeruginosa*; these isolates were confirmed by modified Hodge test and imipenem–ethylene diamine tetra-acetic acid (EDTA) double-disk synergy test [7].

2.7. Genotypic methods for detection of extended-spectrum β-lactamase and AmpC β-lactamase production

2.7.1. Preparation of DNA template

Template DNA was prepared from freshly cultured bacterial isolates by suspending bacterial colonies in 50 μL of molecular-grade water and then heating at 95 °C for 5 min and immediately chilling at 4 °C.

2.7.2. Detection of β-lactamase (bla) genes by PCR

Molecular detection of bla_{CTX-M}, bla_{TEM}, bla_{SHV} and bla_{AmpC} was performed by PCR according to methods described previously with minor modifications (primer profile of bla_{CTX-M}, bla_{TEM}, bla_{SHV} and bla_{AmpC} [5]; thermal profile of bla_{CTX-M} [10]). The primers and cycling conditions for detection of bla_{AmpC} genes were the same as those described by Shahid et al. [11] and Féria et al. [12]. *Klebsiella pneumoniae* ATCC 700603 (ESBL-producer) was used as a quality control strain.

3. Results

Of 87 tracheal aspirate samples from ICU patients (50 male and 37 female), 77 (88.5%) showed significant growth, including 11 with polymicrobial growth. A total of 77 bacterial strains were identified, among which 53 (68.8%) were MDR. Gram-negative bacteria comprised 68 (88.3%) of the total isolates, among which 49 (72.1%) were MDR. Among the 68 Gram-negative isolates, *P. aeruginosa* was the most frequently isolated species with 26 isolates (38.2%), among which 19 (73.1%) were MDR. Gram-positive organisms comprised four (5.2%) of the total isolates, all four (100%) of which were MDR.

Aspergillus fumigatus (5; 6.5%) was the only fungal pathogen identified. On repeated isolation from three consecutive samples, *A. fumigatus* was reported as a pathogen. The results are shown in Table 1.

P. aeruginosa showed high rates of resistance to cefepime (79.2%), ceftazidime (68.5%), gentamicin (71.4%) and ofloxacin (81.7%). Similarly, high rates of resistance were observed among *K. pneumoniae*, *E. coli* and *Acinetobacter baumannii* to cefoxitin, cefotaxime, cefepime and ofloxacin. The results are shown in Fig. 1.

Table 1
Prevalence of microbial isolates and their multidrug resistance (MDR) rates from tracheal aspirates of intensive care unit patients ($n = 87$).

Organism	Frequency (%)	
	Total	MDR
Gram-negative bacteria		
Pseudomonas aeruginosa	26 (33.8)	19 (73.1)
Klebsiella pneumoniae	11 (14.3)	8 (72.7)
Klebsiella oxytoca	4 (5.2)	2 (50.0)
Acinetobacter baumannii	12 (15.6)	10 (83.3)
Escherichia coli	8 (10.4)	5 (62.5)
Citrobacter koseri	7 (9.1)	5 (71.4)
Gram-positive bacteria		
Staphylococcus aureus	4 (5.2)	4 (100)
Fungal pathogens		
Aspergillus fumigatus	5 (6.5)	–
Total	77/87 (88.5)	53/77 (68.8)

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