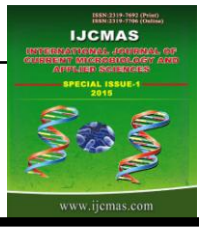
Int.J.Curr.Microbiol.App.Sci (2015) Special Issue-1: 1-9

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Special Issue-1 (2015) pp. 1-9**
http://www.ijcmas.com



Original Research Article

Biofilm Producing Multidrug and Extensive Drug Resistant Bacterial Pathogens from Tracheal Aspirates of Intensive Care Unit Patients – a Threat to Combat

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Keywords

Biofilm, ESBLs, Multidrug resistance, extensive drug resistance, *P. aeruginosa*

A B S T R A C T

Antibiotic resistance among Gram negative bacteria (GNB) causing hospital acquired lower respiratory tract infections (LRTI) poses a grave threat in ICU patients. The objective was to assess the prevalence of Gram negative pathogens in ICU patients and their drug resistance profile. Prevalence of extended spectrum β-lactamases (ESBLs), AmpC and metallo-β-lactamases (MBL) was also assessed. Tracheal aspirates were collected aseptically from 105 ICU patients from February 2012 to November 2013. They were cultured and identified by standard microbiological techniques. Antimicrobial susceptibility was performed according to CLSI guidelines. MBL were detected phenotypically. ESBLs and AmpC were detected both phenotypically and genotypically. GNB constituted 68 (88.3%) of the total isolates, among them *P. aeruginosa* 26 (38.2%) were the most frequently isolated species. Gram-positive organisms constituted 4 (5.1%) of the total isolates and all (100%) of them were MDR. Multidrug resistance, extensive drug resistance and biofilm production was observed in 23.9%, 55.2% and 40.6% isolates respectively. Distribution of $bla_{CTX\text{-}M}$ gene among MDR and XDR isolates was 17.2% and 11.7% respectively while that of bla_{AmpC} was 13.2% and 29.3%. All the MBL (14.4%) producers were XDR. Prevalence of $bla_{CTX\text{-}M}$ and bla_{AmpC} gene in biofilm positive isolates was 10.2% and 27.6% respectively. All the isolates were sensitive to polymixin B and colistin. The average hospital stay of patients positive for biofilm producing isolates was 28 days and was associated with 43.5% mortality. *P. aeruginosa* was found as the predominant pathogen in tracheal aspirate. AmpC producers were the most frequently isolated GNB producing biofilm and showing extensive drug resistance. In view of significant prevalence of biofilm producing MDR amongst Gram-negative organisms in the ICU, regular surveillance of antibiotic susceptibility patterns plays a crucial role in guiding the clinicians for choosing the empirical or directed therapy of infected patients.

Introduction

Tracheostomy is a surgical procedure that creates an opening directly into the trachea to ventilate and aspirate the patient in critical care setting (Pignatti *et al.,* 2009). 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 (Chastre and Fagon, 2002).

The tracheostomized patients are colonized or infected with bacteria either endogenously or exogenously. Exogenous bacteria include *P. aeruginosa*, *A. baumannii*, 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 (Morar *et al.,* 2002). 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 (Fournier and Richet, 2006; Playford *et al.,* 2007).

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 AmpCβ-lactamases and metallo-β-lactamases. 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 (Jacob and Price, 2005).

GNB may also acquire resistance to antibiotics due to permeability barrier of the cell surface in the form of biofilm production. Biofilm-producing organisms are far more resistant to antimicrobial agents than organisms which do not. In some extreme cases, the concentrations of antimicrobials required to achieve bactericidal activity against adherent organisms can be three- to four-fold higher than for those bacteria which do not produce biofilm, depending on the species and drug combination (Dunne, 2002).

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, AmpCβ-lactamase, metallo-β-lactamase and biofilm from tracheal aspirate of patients admitted to ICU.

Materials and Methods

This cross-sectional study was conducted at Jawaharlal Nehru Medical College, AMU, Aligarh from February 2012 to October 2013. A total of 87 tracheal-aspirate samples were included in the study.

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.

Culture of the specimen: The specimens were inoculated on blood agar, MacConkey agar, and chocolate agar plates (Forbes *et al.,* 2007).

Identification and antibiotic susceptibility test: The isolates were identified on the basis of standard microbiological techniques (Forbes *et al.,* 2007). Antibiotic sensitivity test was performed using the Kirby-Bauer disk diffusion method and sensitivity results were interpreted according to CLSI guidelines (CLSI, 2007). Multidrug resistance was defined as resistance to three or more of the antimicrobial agents belonging to different structural classes (Magiorakos *et al.,* 2012).

Phenotypic tests for ESBL production: Isolates were first screened for the production of ESβL by the disc diffusion method (screening test) using cefotaxime, ceftriaxone, cefepime and ceftazidime (CLSI, 2007) and later on confirmed by the cephalosporin/clavulanate combination disk (disk potentiation test) and double disk synergy test (Jacob and Price, 2005). *E. coli* ATCC 25922 (non-ESBL producer) was used as control strain.

Phenotypic methods for AmpC detection: Cefoxitin disks were used to screen AmpC producers, by disk diffusion method (CLSI, 2007). Those isolates which were resistant to cefoxitin were considered as potential AmpC producers.

Phenotypic methods for MβL detection: The isolates were tested for sensitivity to imipenem (10μg) using Kirby-Bauer method as recommended by CLSI (2007). All the isolates with zone of inhibition ≤16 or which demonstrated heaping, or if the zone was >16 but ≤20, were tested for MBL production, however, there is no CLSI guideline for MBL detection available for *Pseudomonas aeruginosa* (CLSI, 2007). These isolates were confirmed by Modified Hodge Test and imipenem- EDTA double disk synergy test (Lee *et al.,* 2002)

Genotypic methods for the detection of ESBL and AmpC production: Detection of *bla* genes by polymerase chain reaction: Molecular detection of $bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV}, bla_{AmpC} was performed by using polymerase chain reaction (PCR) according to methods described previously with minor modifications (Peterson and Bonomo, 2005). The primers and cycling conditions for detection of bla_{AmpC} genes were the same as those described by Shahid *et al.* (2009) and Feria *et al.* (2002). The quality control strain *K. pneumonia* ATCC 700603 (ESBL producer) was used.

Results and Discussion

Out of 87 tracheal aspirate samples, 50 males and 37 females, 77 (88.5%) samples showed significant growth with 11 polymicrobial growth. 77 bacterial strains were identified and among them, 53 (68.8%) were multidrug resistant. Among 77 isolates, Gram-negative bacteria constituted 68 (88.3%) of the total isolates, among which 49 (72.1%) were MDR. Among Gram-negatives, *P. aeruginosa* were the most

frequently isolated species with 26 (38.2%) isolates and among them, 19 (73.1%) were found to be MDR-strains. Gram-positive organisms constituted 4 (5.1%) of the total isolates and all (100%) of them were MDR. Among fungal isolates, *Aspergillus fumigatus* (6.4%) was the most common pathogen. The results are shown in Table 1.

P. aeruginosa showed high rate of resistance to cefexime (81.4%), cefepime (79.2%), ceftazidime (68.5%), gentamicin (71.4%), and ofloxacin (81.7%). Similarly, high rate of resistance was observed among *K. pneumoniae*, *E. coli*, *A. baumannii* to cefoxitin, cefotaxime, cefepime, and ofloxacin. The results are shown in Figure 1.

Staphylococcus aureus showed high rate of resistance to clindamycin (83.3%), ofloxacin (75%), erythromycin (71.7%), gentamycin (67.8%), oxacillin (63.3%) and levofloxacin (72.4%). All the strains of *S. aureus* were sensitive to vancomycin.

ESBL production was confirmed phenotypically in 21(30.9%) isolates and the majority consisted of *K. pneumoniae* with 6 (54.5%), followed by *E. coli* with 03 (37.5%). Out of the 29 (42.2%) AmpCβ-lactamase-positive isolates, *A. baumannii* were the most frequent ones with 07 (58.3%) followed by *P. aeruginosa* with 13 (50%). MBL production was confirmed in 15 (20.95%) bacterial isolates and among them 03 (25%) isolates were *A. baumannii* followed by *P. aeruginosa* 06 (24.3%), *E. coli* 02 (22.5%). The results are shown in Table 2.

Multidrug resistance, extensive drug resistance and biofilm production was observed in 23.9%, 55.2% and 40.6% isolates respectively. This is shown in Table 3. Distribution of $bla_{CTX\text{-}M}$ gene among MDR and XDR isolates was 17.2% and 11.7% respectively while that of bla_{AmpC} was 13.2% and 29.3%. All the MBL (14.4%) producers were XDR. Prevalence of $bla_{CTX\text{-}M}$ and bla_{AmpC} gene in biofilm positive isolates was 10.2% and 27.6% respectively. These results are depicted in Figures 2 and 3.

The results of the study showed high growth rate, which was in accordance with the previous study, which was reported culture positivity of 90% (Jarlier *et al.*, 1988). Polymicrobial growth was observed in more than one-tenth of the cases and the growth of multiple organisms from tracheal specimen has been mentioned in similar studies. 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 (Koirala *et al.*, 2010).

In the present study, 73.1% of *P. aeruginosa* the most predominant isolate of tracheal aspirate, were MDR strains. *P. aeruginosa* showed high rate of resistance to cefexime (81.4%), cefepime (79.2%), ceftazidime (68.5%), gentamicin (71.4%) and ofloxacin (81.7%) which was comparable to the results of two studies. *P. aeruginosa* display an elevated level of drug resistance mechanisms that include production of different types of β-lactamases primarily ESBL, AmpC enzymes and metallo-carbapenemases, aminoglycoside-modifying enzymes, loss of porin proteins, and the presence of efflux pumps like MexAB-Opr M (Picao *et al.*, 2008).

In the present study, 85.4% of *A. baumannii* were MDR. High level of resistance by *A. baumannii* 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, 67% to amikacin) of *A. baumannii* have been observed (Reddy *et al.,* 2010). *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 (Foglia *et al.,* 2007).

In this study, 72.7% of *K. pneumoniae* were MDR strains. These isolates showed high level of resistance against cefotaxime (78.6%), gentamicin (63%), and ofloxacin (70%), which was in harmony with the previous study that reported resistance of 90.5% to cefotaxime, 89% to gentamicin, and 65.8% to ciprofloxacin (Coudron, 2005). High level of drug resistance seen among *K. pneumoniae* is mediated by the production of various types of *β*-lactamases primarily ESBL, AmpC, and metallo-*β*-lactamases along with drug efflux.

ESBL production was confirmed phenotypically in 21(30.9%) isolates and the majority consisted of *K. pneumonia* with 06 (54.5%), followed by *E. coli* 03 (37.5%), *Citrobacter koseri* 03 (28.5%) and *Pseudomonas aeruginosa* 06 (23.1%), which was in contrary to one of the studies conducted in Nepal that showed higher prevalence of *E. coli* with 80% and *K. pneumoniae* with 57.1% (Nseir *et al.,* 2007). Higher rate of ESBL production in *Pseudomonas aeruginosa* has now been increasingly reported due to predominantly occurring SHV- and OXA-type ESBLs.

Out of the 29(42.2%) AmpC *β*-lactamase-positive isolates, *Acinetobacter baumannii* were the most frequent ones with 07 (58.3%) followed by *P. aeruginosa*13 (50%). Plasmidic AmpC genes are derived from the chromosomal AmpC genes of *Enterobacter cloacae*, *Citrobacter freundii*, *Morganella morganii*, and *Hafniaalvei*. Most plasmid-mediated AmpC *β*-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 *β*-lactam therapy (Reddy *et al.,* 2010).

MBL production was confirmed in 15 (20.95%) bacterial isolates and among them, 03 (25%) isolates were *Acinetobacter baumannii* followed by *P. aeruginosa*06 (24.3%), *E. coli* 02 (22.5%). In contrast with this finding, a Korean study reported MBL production in only 14.2% of *A. baumannii* and 11.4% of *P. aeruginosa* (Kumari *et al.,* 2007).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 non-fermenters and members of the family Enterobacteriaceae (Bonomo and Szabo, 2010). MBL-producing bacteria are an increasing public health problem worldwide and mortality rates have been increased due to inadequate empirical therapy (Walsh *et al.,* 2005).

Data obtained in the present work showed that 40.6% of the Gram-negative isolates were biofilm producers and all of them were MDR. All biofilm producing Gram-negative isolates were found to be resistant to most commonly used antibiotics such as penicillin, cephalosporin, aminoglycosides, quinolone and carbapenem group of drugs. This is in agreement with the results of two studies (Nahar *et al.,* 2013).

Table.1 Pattern of microbial isolates from tracheal aspirate of ICU patients

Organisms	**Frequency (%)**	**MDR(%)**
Gram-Negative Bacteria		
Pseudomonas aeruginosa	26(33.7)	19 (73.1)
Klebsiella pneumoniae	11(14.2)	08(72.7)
Klebsiella oxytoca	04(5.1)	02(50)
Acinetobacter baumannii	12 (15.5)	10(83.3)
Escherichia coli	08 (10.3)	05(62.5)
Citrobacter koseri	07(9.1)	05(71.4)
Gram-Positive Bacteria		
Staphylococcus aureus	04 (5.2)	04(100%)
Fungal Pathogens		
Aspergillus fumigatus	05 (6.5)	-
Total	77 (88.5)	53(68.8)

Table.2 Profile of β-lactamase producing bacterial strains from tracheal aspirate of ICU patient

Organisms	**ESBL producers (no. and %)**	**AmpCβ-lactamase producers (no. and %)**	**MBL producers (no. and %)**
P. aeruginosa	06(23.1)	13(50)	06(24.3)
Klebsiella pneumoniae	06(54.5)	03(27.3)	02(19.7)
Klebsiella oxytoca	01(25)	02(50)	01(20)
A. baumannii	02(16.7)	07(58.3)	03(25)
Escherichia coli	03(37.5)	02(25)	02(22.5)
Citrobacter koseri	02(28.5)	03(42.8)	01(14.2)

Table.3 Prevalence of MDR, XDR and Biofilm production among Gram- negative pathogens from ICU patients

Drug Resitance	**Percent of Gram-Negative Isolates**
MDR(Multi-Drug Resistant)	23.9%
XDR(Extensive-Drug Resistant)	55.2%
BFP(Biofilm Positive)	40.6%

Figure.1 Antimicrobial resistance rates (%) for predominant Gram-Negative Bacteria recovered from tracheal aspirate of ICU patients

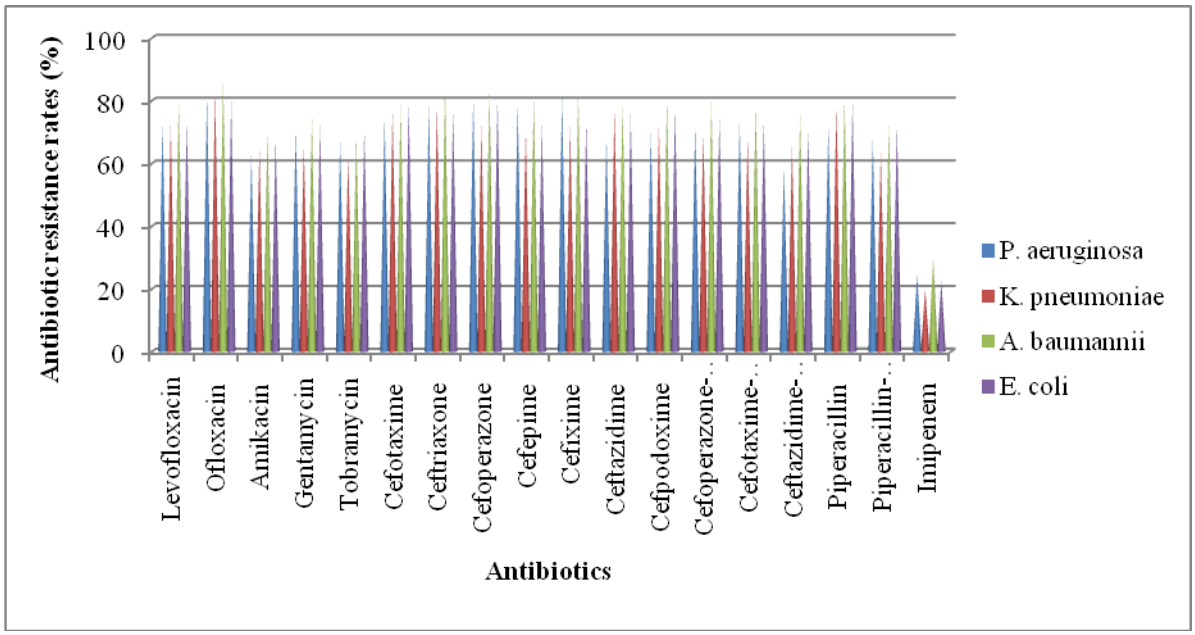


Figure.2 Frequency of $bla_{CTX\text{-}M}$ and bla_{AmpC} gene among β-lactamase-producing bacterial strains from tracheal aspirate of ICU patients

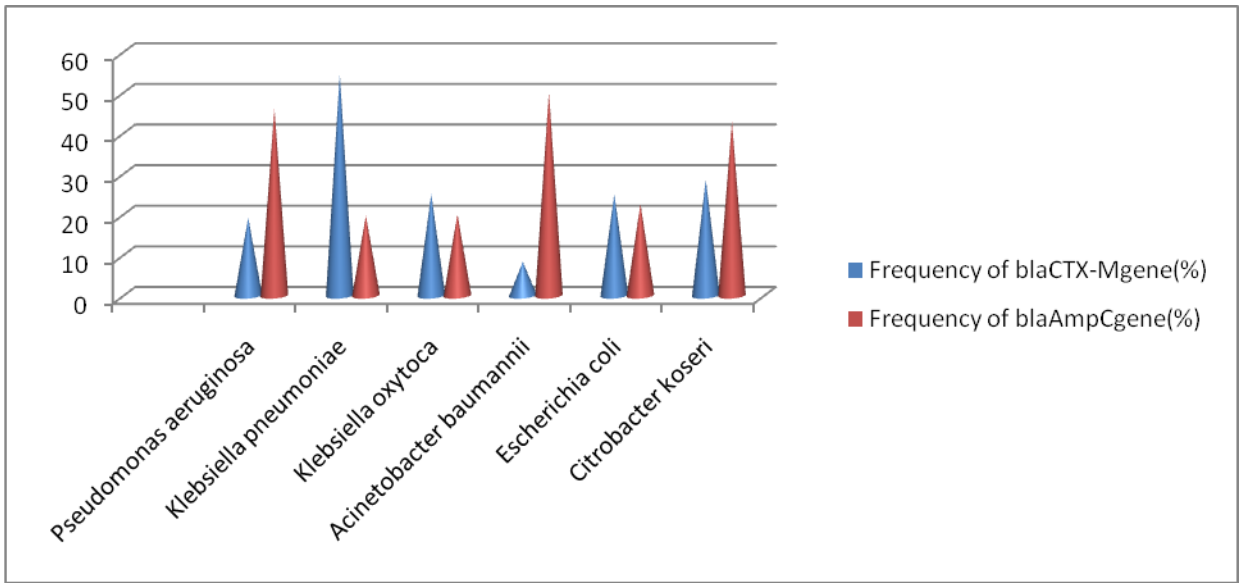
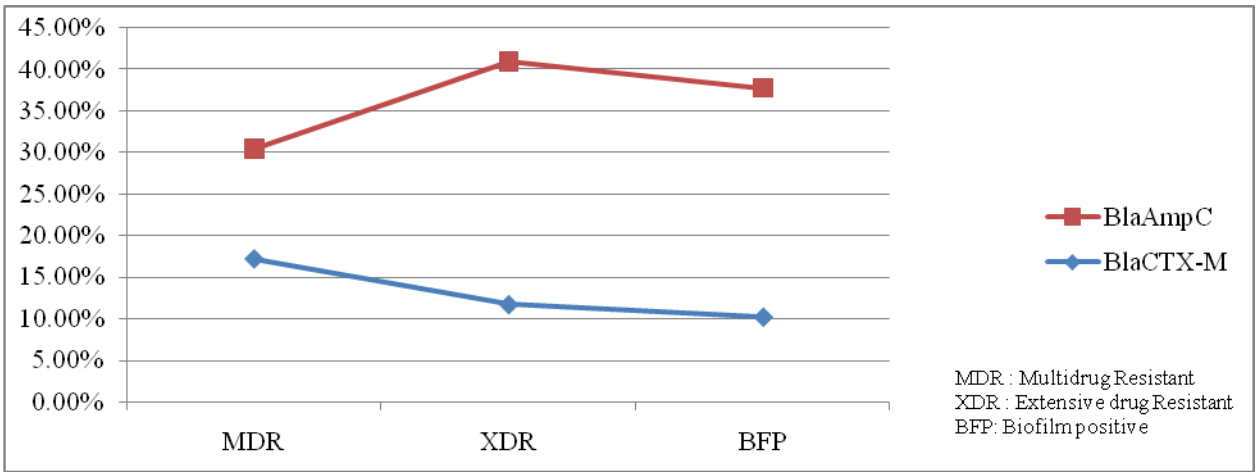


Figure.3 *bla* (*CTX-M, SHV, TEM*) and *bla*(*AmpC*)gene distribution among MDR, XDR and biofilm producing Gram negative pathogens from ICU patients



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 and aminoglycosides. *β*-lactamases and biofilm production confer a high level of resistance to *β*-lactam antibiotics and these traits are usually carried in transferable genes, which are capable of being acquired by normally non-pathogenic bacteria. Therefore, early detection in routine laboratory, immediate infection control, and antibiotic stewardship programs should be implemented in order to limit the spread of MDR and biofilm producing organisms.

References

Bonomo, R.A., Szabo, D. 2006. Mechanisms of multidrug resistance in *Acinetobacter* species and *Pseudomonas aeruginosa. Clin. Infect. Dis.*, 43(2): S49–S56.

Chastre, J., Fagon, Y.J. 2002. Ventilator-associated pneumonia. *Am. J. Respir. Crit. Care Med.*, 165(7): 867–903.

Clinical and Laoratory Standards Institute, 2007. Performance standardsfor antimicrobial susceptibility testing. 17th Informational Supplement CLSI M100-S17, Clinical and Laboratory Standards Institute, Wayne, Pa, USA.

Coudron, P.E. 2005. Inhibitor-based methods for detection of plasmid-mediated AmpC*β*-lactamases in *Klebsiellaspp., Escherichia coli, and Proteus mirabilis. J. Clin. Microbiol.*, 43(8): 4163–416.

Dunne, W.M. Jr. 2002. Bacterial adhesion: Seen any good biofilms lately? *Clin. Microbiol. Rev.*, 15: 155–66.

Feria, C., Ferreira, E., Correia, J.D., Goncalves, J., Canica, M. 2002. Patterns and mechanisms of resistance to beta-lactams and beta-lactamase inhibitors in uropathogenic *Escherichia coli* isolated from dogs in Portugal. *J. Antimicrob. Chemother*., 49: 77–85.

Foglia, E., Meier, M.D., Elward, A. 2007. Ventilator-associatedpneumonia in neonatal and pediatric intensive care unitpatients. *Clin. Microbiol. Rev.*, 20(3): 409–425.

Forbes, B., Sahm, D., Weissfeld, A. 2007. Bailey and Scott's diagnostic microbiology, 12th edn., Mosby, Texas.

Fournier, P.E., Richet, H. 2006. The epidemiology and control of *Acinetobacter baumannii* in health care facilities. *Clin. Infect. Dis.*, 42(5): 692–699.

Jacob, G.A., Price, L.S.M. 2005. The new beta-lactamases. *N. Eng. J. Med.*, 352(4): 380–391.

Jarlier, V., Nicolas, M.H., Fournier, G., Philippon, A. 1988. Extended-spectrum beta lactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae: hospital prevelance and susceptibility patterns. *Rev. Infect. Dis*., 10: 867–878.

Koirala, P., Bhatta, D.R., Ghimire, P., Pokhrel, B.M., Devkota, U. 2010. Bacteriological profile of tracheal aspirates of thepatients attending a neuro-hospital of Nepal. *Int. J. Adv. Life Sci.*, 4: 60–65.

Kumari, H.B.V., Nagarathna, S., Chandramuki, A., 2007. Antimicrobial resistance pattern among aerobic gram-negative bacilliof lower respiratory tract specimens of intensive care unitpatients in a neurocentre. *Indian J Chest Dis. Allied Sci.*, 49(1): 19–22.

Lee, K., Lim, J.B., Yum, J.H., Yong, D., Chong, Y., Kim, J.M. 2002. blaVIM-2 cassette-containing novel integrons in metallo-β-lactamase-producing Pseudomonas aeruginosa and Pseudomonas putida isolates disseminated in a Korean hospital. *Antimicrob. Agents Chemother.*, 46: 1053–1058.

Magiorakos, A., Srinivasan, A., Carey, R. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standarddefinitions for acquired resistance. *Clin. Microbiol. Infect.*, 18(3); 268–281.

Morar, P., Singh, V., Makura, Z. 2002. Oropharyngeal carriage and lower airway colonisation/infection in 45 tracheotomisedchildren. *Thorax*, 57(12): 1015–1020.

Nahar, A., Anwar, S., Miah, M.R.A. 2013. Association of biofilm formation with antimicrobial resistance among the *Acinetobacter* species in a tertiary care hospital in Bangladesh. *J. Med.*, 14: 28–32.

Nseir, S., Pompeo, C.D., Pronnier, P. 2002. Nosocomial tracheobronchitisin mechanically ventilated patients: incidence, aetiology and outcome. *Eur. Respir. J.*, 20(6): 1483–1489.

Peterson, D.L., Bonomo, R.A. 2005. Extended spectrum beta lactamases: a clinical update. *Clin. Microbiol. Rev.*, 18: 657–686.

Picao, R.C., Andrade, S.S., Nicoletti, A.G. 2008. Metallo-*β*-lactamase detection: comparative evaluation of double-disksynergy versus combined disk tests for IMP-, GIM-, SIM-, SPM-, or VIM-producing isolates. *J. Clin. Microbiol.*, 46(6): 2028–2037.

Pignatti, P., Balestrino, A., Herr, C. 2009. Tracheostomy and related host-pathogen interaction are associated with airway inflammation as characterized by tracheal aspirate analysis. *Res. Med.*, 103(2): 201–208.

Playford, E.G., Craig, J., Iredell, J. 2007. Carbapenem resistant *Acinetobacter baumannii* in intensive care unit patients: risk factors for acquisition, infection and their consequences. *J. Hosp. Infect.*, 65(3): 204–211.

Reddy, T., Chopra, T., Marchaim, D. 2010. Trends in antimicrobial resistance of *Acinetobacter baumannii* isolates from a Metropolitan Detroit health system. *Antimicrob. Agents Chemother.*, 54(5): 2235–2238.

Shahid, M., Malik, A., Adil, M., Jahan, N., Malik, R. 2009. Comparison of beta-lactamase genes in clinical and food bacterial isolates in India. *J. Infect. Dev. Ctries.*, 3: 593–598.

Walsh, T.R., Toleman, M.A., Poirel, L., Nordmann, P. 2005. Metallo-*β*-lactamases: the quiet before the storm? *Clin. Microbiol. Rev.*, 18(2): 306–32.