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Full Length Research Paper

Microbiological and molecular detection of *Pseudomonas aeruginosa* in nasogastric tube fed geriatric patients

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Nasogastric tube feeding is the most common and oldest form of interventional feeding. The oropharynx of nasogastric tube fed elderly patients provides an ecosystem that promotes the colonization of *Pseudomonas aeruginosa*. The study was conducted to determine the incidence of *P. aeruginosa* isolation from the oropharynx of nasogastric tube fed elderly patients and explore its antibiotic sensitivity. The work was carried on 60 geriatric patients who were admitted in the surgical and medical ICUs of El-Demerdash Hospital. The patients were suffering from an active disease and require close medical supervision, 30 control subjects matched orally fed who resident in the same wards were included. Sterile cotton swab was applied to the dorsum of the tongue rubbing buccal mucosa and the oropharyngeal region, for microbiological culture and DNA extraction of *P. aeruginosa* for real time polymerase reaction. Incidence of *P. aeruginosa* isolation in the nasogastric tube fed patients was 43.3% in comparison to 3.3% in the control orally fed group. 57% of these patient sample were reported negative by both culture and real time polymerase chain reaction (PCR), 40% were positive by both methods, none was positive only by culture whereas 3% were positive only by real time PCR. The only single positive case in the control group was positive by both methods. The antibiotic sensitivity of the isolated *P. aeruginosa* in the patient group was detected according to the Clinical laboratory standard institute guidelines (CLSI, 2007) and reported multidrug resistant *P. aeruginosa* as 15% whereas the single positive control case was sensitive to all tested antibiotics. There is an urgent need to develop and investigate new effective antiseptic compounds and adopt oral health care techniques for elderly patients with nasogastric tube feeding.

Key words: *Pseudomonas aeruginosa*, nasogastric tube (NGT), clinical laboratory standard institute (CLSI).

INTRODUCTION

Tube feeding is used to supply nutritional formula to immobilized patients. It is favored over parenteral nutrition in most critically ill patients because the former is thought to preserve the integrity of the gut and cause fewer infections complications (Nuutinen, 1997). As with most therapies, however, tube feeding has associated risks. The most dreaded complication of tube feeding is tracheobronchial aspiration of gastric contents (Metheny et al., 2006). Among pathogens conferring risk for severe aspiration pneumonia is gram negative bacteria (GNB) and *Staphylococcus aureus (S. aureus)* (Marik, 2001). GNB are found in the oropharynx of up to 71% of nasogastric tube (NGT) fed patients and in 44% of those fed by percutaneous enterogastric tube compared with only 7.5% of their orally fed counterparts (Leibovitz et al., 2003). As the organism adhere to a wet environmental surface it produce a slimy, sticky polysaccharide substance and became a part of the biofilm growing on the polyvinyl chloride nasogastric tube (Kate, 2003). *P. aeruginosa* isolates from the oropharynx were less

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sensitive to most antibiotics (Leibovitz et al., 2003). *Pseudomonas aeruginosa* infections in the ICU are a constant concern (Souli et al., 2008). Specific interaction between both patient colonization pressure and selective antibiotic pressure is the most relevant factor for *P. aeruginosa* acquistation on an ICU. This suggests that combined efforts are needed against both factors to decrease colonization with *P. aeruginosa* (Boyer et al., 2011). Prevention and control of biofilms on medical devices require consideration of the unique and tenacious nature of biofilms. Multiple intervention strategies include preventing initial colonization of the device, minimizing attachment of bacterial cells to the device, penetrating the biofilm matrix to kill the bacterial cells, and removing the device from the patient. Eventually, removal of the medical device may be necessary because a biofilm can cause acute or chronic infectious disease. The ability of biofilms to resist disinfectants and antibiotics makes them a public health problem. Biofilms are an important cause of nosocomial infections, because once established, the bacteria harbored inside are less exposed to a patient's immune response and are less susceptible to antibiotics (Solseng et al., 2008).

The intensive care unit (ICU) is the epicenter of infections mainly due to its extremely vulnerable population of critically ill patients, and the high use of invasive procedures. These infections are associated with an important rise in morbidity, mortality and health care costs. The additional problem of multidrug-resistant pathogen boasts the adverse impact of infections in ICUs. Several factors influence the rapid spread of multidrug resistant pathogens in the ICU, new mutations, selection of resistant strains, and suboptimal infection control (Brusselaers et al., 2011). Thus, the aim of this study was to reconfirm the continual high incidence of *P. aeruginosa* isolations from the oropharynx of NGT fed elderly patients, determine its exact incidence of isolation among the same selected group in an educational tertiary care hospital in Egypt using both microbiological and molecular techniques as this could constitute a potential reservoir for spread of multidrug resistant *P. aeruginosa* in hospitals in Egypt.

MATERIALS AND METHODS

Prospective study was conducted on a group of 60 geriatric patients of age between 68 and 83 years old from November, 2010 to July, 2011. They were admitted in the surgical and medical ICUs of El-Demerdash Hospital. The patients were suffering from an active disease and require close medical supervision as stroke, dementia, ischemic heart disease, diabetes mellitus and chronic obstructive pulmonary disease (COPD). All eligible patients had nasogastric tube (NGT) feeding for at least 2 weeks. Those patients were under oral hygienic for the nasogastric tube, that was performed by cleansing the oral cavity before meal three times a day with lemon-glycerin wadding sticks impregnated with a solution of glycerin-citric acid, lemon flavoring and sodium benzoate 0.1% (Warner, 1986). NGTs in use were made from polyvinyl chloride.

30 control subjects matched orally fed patients with no swallowing disturbances who resident in the same wards were included in the study; these patients had received routine oral hygiene with brush done by the nurses in the ICU. From both group, patients who had received an antibiotic treatment up to 2 weeks before the study, patients with advanced cancer and patients who had received chemotherapy or radiotherapy to the neck were excluded.

Sterile cotton swab was applied to the dorsum of the tongue rubbing buccal mucosa and the oropharyngeal region (BBL culture swab cat #22 00 99). The samples were collected in the morning before breakfast and daily oral cleaning procedures. The swab was placed in transport liquid staurt media. The sample was vortexed in the liquid staurt medium and two aliquots of 200 ml each were collected from the medium. First aliquot was used for microbiological culture and the second aliquot was stored at 80℃ prior to DNA extraction for real time polymerase reaction (PCR).

Culture and identification of the bacteria

50 µl of each of the vortexed sixty samples were inoculated onto Mac Conkey agar plate and (100 µl) into 4 ml of cetrimide selective broth media for *Pseudomonas aeruginosa* (Fluka Biochemika, Bucks, Switzerland) and incubated for at least 24 h at 37℃ at ambient atmosphere before examination. The positive growth of both the cetrimide and non lactose fermenting colonies on the Mac Conkey was subcultured on sheep blood agar plate by applying 50 ul of the positive cetrimide broth and the suspicious non lactose fermenting colonies of the Mac Conkey plate and the plates were incubated for 24 h at 37 to 42℃. The colonies were identified by oxidase test, API-20 NE strips (BioMerieux vitek, USA) and colistin disc sensitivity.

The antibiotic susceptibility of *P. aeruginosa* by disc diffusion method: The susceptibility of *P. aeruginosa* isolated from the patients to antibiotics were determined by disc diffusion method to Peperacillin (100 µg) Peperacillin-tazobactam (100/10 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Cefepime (30 µg), Imipenem (10 µg), Meropenem (10 µg), Aztreonam (30 µg), Gentamicin (10 µg), Amikacin (30 µg), Ciproflaxin (5 µg) as proposed by CLSI (2007) that recommends a zone diameter for Piperacillin (100 µg) ≥ 18 mm, Piperacillin/tazobactam (100/10 µg) ≥ 18 mm, Ceftazidime (30 µg) ≥ 18_, Cefepime (30 µg) ≥ 18 mm, Ceftriaxone (30 µg) ≥ 21 mm, Imipencin (10 µg) ≥ 16 mm, Meroponem (10 µg) ≥ 16 mm, Aztreonam (30 µg) ≥ 22 mm, Gentamicin (10 µg) ≥ 15 mm, Amikacin (30 µg) ≥ 17 mm, Ciprofloxacin (5 µg) ≥ 21 mm and Colistin sulphate (10 µg) ≥11 mm for *Pseudomonas aeruginosa* to be recorded as sensitive (Table 4).

Real time polymerase reaction

Light cycler FastStart DNA master SYBR green 1 that is a ready to use PCR reaction mix (cat no. 03003230001) designed specifically for real time PCR assays using SYBR green 1 detection format on the light cycler carousel based system (Roche). It is used to perform hot start PCR in 20 µl glass capillaries. Hot start PCR has been shown to significantly improve the specificity and sensitivity of PCR by minimizing the formation of non specific amplification products at the beginning of the reaction (PCR manual, Roche diagnostics, 1999).

Specimen preparation and DNA extraction

DNA was extracted using the protocol generic 2.0.1 on the biomerieux easy MAG Nuclisens extractor (biomerieux, marcy-l'etoile, france) in which each sample was combined with 500 µl of

Table 1. Demographic data of the studied groups.

Studied group	NGT –fed patients (60)	Orally fed patients(30)	P value
Age	78 ± 9	81 ± 9	>0.05
Stroke	34 (56.6%)	16 (54.3%)	>0.05
Dementia	11(18%)	5 (16.7%)	>0.05
Ischemic heart disease	45 (75%)	23 (76.7 %)	>0.05
COPD	12 (20%)	7 (23%)	>0.05
Diabetes controlled uncontrolled	12 (20%) 7 (11.7%) 5 (8.4%)	1 (3.3 %) 0 1	>0.05
Incidence of *Pseudomonas* isolation	26 (43.3%)	1 (3.3%)	<0.05

Table 2. Direct culture compared to Real time PCR for *p aeruginosa* detection in the patient group.

Total number of patients	Direct culture				PCR			
	+ve		-ve		+ve		-ve	
60	No.	%	No.	%	No.	%	No.	%
	24	40	36	60	26	43	34	57

2x buffer B (143 mMtris pH8.0), 143 mM Nacl, 14 mM EDTA and 5.7% SDS), 500 µl phenol chloroform and approximately 0.25 zirconinum beads (0.1 mm, biospec products inc.). Samples were centrifuged 16,000×g for 3 times. The DNA from the supernatant was precipitated using 7.5 M ammonium acetate (160 µl) and isoproponol (560 µl). This mixture was then centrifuged (16,000×g) for twenty minutes and decanted. The DNA pellet was washed with 1ml of 70% ethanol and centrifuged for five minutes to ensure recovery of the DNA pellet. The DNA pellet was air dried and resuspended in 50 µl of TE (10 m M Tris pH 8.0), 1 mM EDTA. All extractions included a negative extraction control where buffer only was extracted in parallel with specimens. A sample of 5 µl was taken as a template for PCR.

Negative control

A negative control was used with the samples. It was prepared by replacing the template DNA with PCR grade water. Positive control was prepared by reconstituted control DNA prepared from *Pseudomonas aeruginosa* ATCC (10145).

RESULTS

The study group comprised of 60 elderly patients between 68 and 83 years old with mean +SD (78±9). Their demographic data were matched with 30 control orally fed patients with no significant difference (Table 1). The incidence of *P. aeruginosa* isolation in the nasogastric tube fed patients was 43.3% in comparison to 3.3% in the orally fed patients which shows significant difference between them. Thirty four (34) swab samples were negative by both direct culture and real time PCR for *P. aeruginosa* detection (57%), 24 patient swab samples were positive by both methods (40%), none was positive only by culture (0%) and 2 patient swab sample were positive only by real time PCR (3%). Thus PCR was able to detect *P. aeruginosa* in 43% of the cases. The two samples that were real time PCR positive and culture negative on review their results on the selective cetrimide broth media; they provided a final culture result as positive for *P. aeruginosa* at 48 h (Table 2). The sensitivity, specificity, positive predictive value and negative predictive value of PCR were 100, 94, 92 and 100%, respectively (Table 3).

The isolates that were reported as multidrug resistant, *P. aeruginosa* (MDRPA) were four out of the 26 isolated organisms (15%) and this, according to the CDC definition of MDRP that was established; isolates of *P. aeruginosa* intermediate or resistant to at least three drugs in the following classes: beta lactam, carbapenem, aminoglycosides and fluoroquinolons are considered resistant. The single positive control sample was susceptible to most of the antibiotic tested. Statistical processing was performed by using SPSS software (SPSS Inc.). Chi-square test was used for comparative studies; $P<0.05$ was considered significant.

DISCUSSION

NGT feeding is the most common and oldest form of interventional feeding. The benefits of this method are that little skill is required for tube replacement and it enables early commencement of tuboentral feeding that maintains intestinal function (Esparza et al., 2001). As

Table 3. Sensitivity, specificity, positive and negative predictive values of real time PCR.

Parameter	Value (%)
Sensitivity [1]	100
Specificity [2]	94
Positive predictive value [3]	92
Negative predictive value [4]	100

[1]Data are no. of both culture and PCR-positive results/no. of direct culture-positive results. Sensitivity = True Positive / (True Positive + False Negative).[2]Data are no. of both culture and PCR-negative results/no of direct culture-negative results. Specificity = True Negative / (False Positive + True Negative). [3]Data are no. of both direct culture and PCR- positive results/no. of PCR- positive results. Positive predictive value= no. of both culture and PCR- positive results/no. of PCR- positive results. Positive predictive value= True Positive / (True Positive + False Positive). [4]Data are no. of both direct culture and PCR- negative results/no. of PCR-negative results. Negative predictive value= no. of both culture and PCR- negative results/no. of PCR-negative results. Negative predictive value = True Negative / (False Negative + True Negative).

Table 4. Percentage of antibiotic sensitivity to the isolated *P. aeruginosa* in the patient group.

Antimicrobial agent	Disk content (µg)	Zone diameter nearest whole (mm)			Number of samples showed sensitivity	Percentage of sensitivity (%)
		R	I	S		
Piperacillin	100	≤17	-	≥18	23	88
Piperacillin tazobactam	100µg/10µg	≤17	-	≥18	24	92
Ceftazidime	30	≤14	15-17	≥18	23	88
Cefepime	30	≤14	15-17	≥18	23	88
Imipenem	10	≤13	14-15	≥16	21	81
Meropenem	10	≤13	14-15	≥16	20	77
Aztreonam	30	≤15	16-21	≥22	22	85
Gentamicin	10	≤12	13-14	≥15	21	81
Amikacin	30	≤14	15-16	≥17	22	85
Ciprofloxacin	5	≤15	16-20	≥21	22	85
Colisim Sulphate	10	≤9	-	≥11	26	100

well as feeding tubes are valuable assets in the rehabilitation of adult's patients with dysphasia, in addition they may be placed in response to perceived risks of compromised airway or insufficient nutrient intake (Crary and Groher, 2006). Aspiration of or pharyngeal contents is a constant threat for elderly patients fed via a nasogastric tube (NGT). Colonization of or pharynx of these patients by a pathological flora and the development of biofilms on the feeding tube has been documented. In addition, the presence of the NGT may interfere with the gastro-esophageal sphincter, leading to gastro- esophageal reflux. Thus, the passage of pathogenic bacteria in both directions is facilitated (Segal et al., 2006).

Leibovitz et al. (2003) performed a bacteriological study on 90 patients fed by nasogastric tube compared to a control group of elderly patients in long term care who are fed orally. The study revealed a significantly high prevalence of gram negative bacteria in nasogastric tube fed patient which was about 73% and emphasized that *Pseudomonas aeruginosa* and *klebsiella pneumonia* were commonly and exclusively isolated from the oral flora of the nasogastric tube fed patients, these results were close to the findings detected in our study. This study revealed that 43.3% of the studied patients with nasogastric tube were harbouring *P. aeruginosa* in their oropharynx in comparison to 3.3% in the control group with significant difference between the two studied groups (Table 5). The difference in the percentage between this recent study and other study may be attributed to the difference in the aim of the study; this study is aimed to determine the incidence of *P. aeruginosa* in the oropharynx of the nasogastric tube fed patients and not all the gram negative bacteria. This was also confirmed by Leibovitz et al. (2003) showed that tuboenteral feeding in elderly patients in associated with pathogenic colonization of the oropharynx and that *P. aeruginosa* was cultured from 31% of patients on NGT feeding.

The present study revealed that one out of 30 patients

Table 5. Incidence of *Pseudomonas* in tube feeding.

Variable	Nasogastric tube	Control
Total number	60	30
Number of *Pseudomonas* infection	26	1
Incidence of *Pseudomonas*	43%	3.3 %
P value	<0.05	

of the control group with no tuboentral feeding grew *P. aeruginosa*, and on review of this case, we found that he was uncontrolled diabetic patient with respiratory complications. This may be the cause of growing *P. aeruginosa* from his oropharynx, or it may be hospital acquired by the hands of health care workers who are lacking standards of care, and paying insufficient attention to the preventive oral health of the tuboentral feeding patients that were in the same ward. Bengmark (2004) mentioned that recognized hospital acquired infections are an important determinant of outcome in patients who require intensive care unit (ICU) admission. The source of respiratory tract colonization can be exogenous from the hands of the health care workers or the patient's skin, but it can also be endogenous, such as from the intestine, the oropharynx and the gastric compartment, followed by retrograde contamination. Bacterial proliferation in the stomach is potentially enhanced by entral nutrition in combination with administration of antiulcer prophylaxis drugs.

Slekovec et al. (2010) evaluated the necessity of screening samples to assess *P. aeruginosa* endemicity in ICU. They found that 146 patient samples were positive for *P. aeruginosa* among 754 patients included giving an average incidence of 19.4 per 100 patients. Thirty five were imported and 111 ICU acquired. Todar's (2007) stated that according to the centers for disease control and prevention (CDC), *P. aeruginosa* is the fourth most commonly isolated nosocomial pathogen, accounting for 10.1% of all hospital acquired infections.

Kate (2003) stated that *P. aeruginosa* obtained from the tube fed patients were less susceptible to Amikacin but had similar degrees of resistance to tazobactem-piperacillin, ceftazidime and imipenem, if compared to a reference sample of *P. aeruginosa* cultured from sputum samples obtained from residents for the same facility. In this study, 19% (5 cases) of the isolated *P. aeruginosa* were resistant to imipenem, 15% (4 cases) of the isolates were resistant to Amikacin, Aztreonam, and Ciprofloxacin. 12% of the isolates were resistant to piperacillin, ceftazidime, and cefepime, while 23% of them were resistant to meropenem. All the isolated *P. aeruginosa* showed no resistance to the colistin sulphate, in addition 15% (4 samples) were reported as multidrug resistant *P. aeruginosa* (MDRPA).

Furtado et al. (2010) reported that imipenem resistant *P. aeruginosa* is a leading cause of hospital acquired pneumonia. Previously, leibovitz et al. (2003) mentioned that *P. aeruginosa* isolated from the oropharynx showed the highest susceptibility rates registered for tazobactem-piperacillin with 89% of the isolates being susceptible, followed by ceftazidime and imipenem. These results are in consistence with the results of this current study, in which the highest susceptibility rates (92%) were registered for piperacillin-tazobactem followed by third and fourth generation cephalosporins represented in ceftazidime and cefepime with (88%). This was also emphasized by Juan and Oliver (2010) who mentioned that *P. aeruginosa* pathogen has high intrinsic antibiotic resistance, so this together with the extraordinary capacity for acquiring additional resistances through chromosomal mutations, determines a major threat for antimicrobial therapy in hospitals worldwide. Even more concerning is the increasing detection of multiple antimicrobial resistance determinants in the micro-organism, frequently located on integrons, acquired by horizontal transfer through plasmids and/or transposons. Among these mechanisms, the carbapenemases are particularly relevant, due to the wide spectrum of antibiotics affected.

Boyer et al. (2011) found that by univariate analysis, the presence of an invasive device (nasogastric tube), and previous patient colonization pressure on the same ward from the ICU and shared room were significantly associated with *P. aeruginosa* acquisition. Multivariate analysis revealed that the presence of a nasogastric tube was independently associated with *P. aeruginosa.* In addition, the interaction between antibiotics inactive against *P. aeruginosa* and the patient colonization pressure was also significant.

Manoharan et al. (2010) stated that metallo-beta-lactamases (MBLs) production is a significant problem in hospital isolated *P. aeruginosa* and all imipenem-resistant isolates had detectable MBLs, however there are resistance mechanisms involved, such as permeability mutations via the loss of porins or the up-regulation of effux systems.

In this present study, 23% of the isolates of *P. aeruginosa* (6 cases) were resistant to meropenem by the disc diffusion method. In this work, sensitivity, specificity, positive and negative predictive values of real time PCR for *P. aeruginosa* were 100, 94, 92 and 100%, respectively. Real time PCR was able to detect two samples to be positive after they failed to be detected on

the Mac Conkey plate but detected after growth on the on cetrimide selective broth media.

Deschagt et al. (2010) mentioned that taking culture is the gold standard; the PCR had a sensitivity of 90%, a specificity of 85% and positive predictive value of 77% and a negative predictive value of 99%. The difference in the percentage between this present study and the previous study, maybe attributed to the difference in the number of studied cases, type of samples, method of DNA extraction and sample processing which (Deschagt et al., 2010) were comparing the sensitivity of micro-biological culture and quantitative PCR (qPCR) using light cycle Taq Man Master mix for the detection of *P. aeruginosa* in respiratory samples of not chronically infected CF patients.

In spite of the application of special oral hygiene to nasogastric tube fed patients in this study, there was a significant high incidence of *P. aeruginosa* isolation in comparison to the control group. This may be due to several factors such as the papillary structure of the dorsum of the tongue, the lack of mastication and swallowing (eliminating their mechanical cleansing effect), and the tube itself. *P. aeruginosa* itself is a well-known biofilm-producing microorganism (Leibovitz, 2003). In addition, it may be attributed to the type of oropharyngeal cleaning used in the study as two clinical studies (one in ICU and another in a multi-nursing home setting using random controls) have shown a decrease in rates of pneumonia, including VAP, upon implementation of a comprehensive oral-hygiene program for patients or residents.

Moreover, this study did not monitor the secure and infection control care for the tube itself, thus this factor may play a role in this high incidence. Thus, the urgent need to develop and investigate new effective antiseptic compounds and adopt oral health care techniques taking into consideration infection control guidelines for elderly patients with nasogastric tube feeding to reduce *P. aeruginosa* oropharyngeal infection and the subsequent occurrence of pneumonia with resistant isolates is mandatory recommended.

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REFERENCES

Bengmark S (2004). Symbiotics to strengthen gut barrier function and reduce morbidity in critically ill patients. Clin. Nutr., 23(4): 441-445.

Boyer A, Doussau A, Thiebault R, Venier AG, Tran V, Boulestreau H, Bebear C, Vargas F, Hilbert G, Gruson D, Rogues AM (2011). *Pseudomonas aer*uginosa acquistation on an intensive care unit: Relationship between antibiotic selective pressure and patients environment. Critical care, 15:R55.

Clinical and laboratory standards institute (2007). Performance standards for antimicrobial susceptibility testing. 17th information supplement M100-S17. Vol.27 Clinical and Laboratory standards institute, Wayne, PA.

Crary AM, Groher EM (2006.) Reinstituting oral feeding in tube fed adults patients with Dysphagia. Nutr. Clin. Pract., 21(6): 576-586.

Deschagt P, Scheistraeta P, lopes dos G, Santiago S, Van Simaey L, Haerynck F, Van daele S, De Wachter E (2010). Comparison of the culture, PCR and qPCR for the detection of *Pseudomonas Aeruginosa* in not chronically infected cystic fibrosis patients. BMC Microbiol., 10: 245.

Esparza J, Boivin MA, Hartshorne MF, Levy H (2001). Equal aspiration rates in gastrically and transpylorically fed critically ill patients. Intensive care, Med., 27: 660-664.

Furtado GH, Gales AC, Perdiz LB, Santos AE. Wey SB, Medeiros EA (2010). Imipenem resistant *Pseudomonas aeruginosa* is a leading cause of hospital acquired pneumonia. Anaesth, Intensive care, 38(6): 987-988.

Juan NC, Oliver A (2010). Carbapenemases in *Pseudomonas* spp. Enferm Infect. Microbiol. Clin., 28: 19-28.

Kate T (2003). Nasogastric tubes may harbor pathogens. American. Society of health system pharmacist. News article, 60: 1516.

Leibovitz A, Dan M, Zinger J, Carmeli Y, Habot B, Segal R (2003).*Pseudomonas aeruginosa* and the or pharyngeal ecosystem of tube fed patients. Emer. Infect. Dis., 9: 956-959.

Manoharan A, Chatterjee S, Mathai D (2010). Detection and characterization of metallo beta lactamases producing *Pseudomonas Aeruginosa*. Indian J. Med. Microbiol., 28: 241-244.

Marik PE (2001). Aspiration pneumonitis and aspiration pneumonia. N. Engl. J. Med., 344: 665-671.

Metheny NA, Clouse RE, Chang YH, Stewart BJ, Oliver DA, Kollef MH (2006). Tracheobranchial aspiration of gastric contents in critically ill tube fed patients: frequency outcomes and risk factors. Crit. Care Med., 34:1007-1005.

Nuutinen L (1997). Entral or parenteral nutrition? Acta Anaesthesiol Scand Suppl., 110: 141-142.

PCR manual (1999). Roche diagnostics 2nd edition, 2: 52-58.

Segal R, Pogoreliuk I, Dan M, Baumoehl Y, Leibovitz A (2006). Gastric microbiata in elderly patients fed via nasogastric tubes for prolonged periods 63(1): 79-83.

Slekovec C, Navellou JC, Blasco G, Thouverez M, Bertrand X, Talon D (2010). Is surveillance of *Pseudomonas aeruginosa* carriage in intensive care units useful? Ann Fr Anesth. Reanim, 29(4): 279-82.

Souli M, Galani I, Giamarellou H (2008). Emergence of extensively drug resistant and pandrug–resistant gram negative bacilli in Europe. Euro Surveill 13: pii: 19045.

Todar's online textbook of bacteriology 2007 Accessed 29/2/2007. Available at www.text book of bacteriology.net.

Warner LA (1986). Lemon-glycerine swabs should be used for routine oral care. Crit. care nurse, 6: 82-83.

Brusselaers N, vogeaers D, Blot S (2011). The rising problem of antimicrobial resistance in intensive care unit. Annals of intensive care unit 1:47 doi: 10.1186/2110-582.