MICROBIOLOGICAL FLORA AND THEIR ANTIBIOTIC SUSCEPTIBILITY- A STUDY UNDERTAKEN WITH ENDOTRACHEAL TUBE TIPS AND ENDOTRACHEAL ASPIRATES IN A TERTIARY CARE HOSPITAL IN KOLKATA

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ABSTRACT

BACKGROUND

Endotracheal (ET) tubes are life saving devices on one hand and life takers on the other. This study aims to find out how morbidity in the form of nosocomial pneumonia can be predicted to save patients and to minimise expenditure.

MATERIALS AND METHODS

This was a descriptive study. ET tube tips, tracheal aspirates and laryngeal aspirates were collected and tested from patients intubated for a minimum of 48 hours. 31 such samples were processed by culture in MacConkey Agar and Blood Agar media. Growth was observed.

RESULTS

The results showed the growth of *Klebsiella Pneumoniae* (54.1%) and *Acinetobacter baumannii (*33.3%). These findings are similar to several other studies in different parts of India e.g. by Chandra Mouli et al, Dipti Chandra et al 2017 etc. and earlier by Elek and Conen in 1957. Antibiogram by Kirby Bauer method showed that all the organisms were multidrug resistant.[1,2,3,4]

CONCLUSION

This study reinforces the finding that ET tubes are a very important cause of pneumonia. Gram negative organisms in this study are the causative agents. Most of the organisms were sensitive to Colistin (except *Proteus mirabilis,* which is known to be intrinsically resistant). Moreover, the medical fraternity must be aware that on one hand the infections need immediate and correct intervention and on the other hand the resistance pattern shows that the choice of drugs is extremely limited and only the most toxic of them remain useful. Thus, the management of such cases has to be very carefully planned and balanced taking into consideration the immune status, age, co-morbidities and economic status of each patient.

KEY WORDS

Endotracheal Tube, Nosocomial Infection, Multidrug Resistant, Co-Morbidity, Virulence Factors, NICU and PICU.

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BACKGROUND

Personnel related to the medical profession are well aware that endotracheal tubes are mandatory in any instance requiring assisted respiration, which may be iatrogenic (as in general anaesthesia, collection of aspirate, biopsy etc.) or in diseases. On one hand intubation may be a life saver, i.e. an absolute necessity when the patient cannot breathe and on the other hand it is (more often than not) a major cause of nosocomial infection. The patients are ultimately saved at the cost of skilled professional help, a magnum expenditure and a huge toll on their already old and feeble bodies.

The cycle is usually the same severely ill patient followed by intubation, followed by nosocomial infection with MDR organism septicaemia. The fate is either, patients succumb (majority) or lucky ones respond and get well.[5,6,7]

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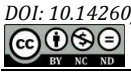

The huge increase in hospital stay followed by increase in expenditure and many a time a fatal outcome at the end of it all lead us to undertake this study.

MATERIALS AND METHODS

A descriptive study was performed over six months from January 2017 to June 2017.

Samples

ET tubes- tips and aspirates. Samples were only those whose ET tubes were present in situ for 48 hours or more. Thirty-one such samples were obtained. Each sample was cultured aerobically in Blood agar and MacConkey agar. The plates were examined after 48 hours. Twenty-four samples were culture positive. Examination of colony characters, Gram stain and motility tests were done from the isolates. Biochemical reactions routinely performed in the laboratory including oxidation fermentation reaction in Hugh-Leifson media, catalase test, oxidase test, indole reaction, glucose, lactose mannitol and sucrose fermentation, citrate utilisation test, growth in triple sugar iron agar media, lysine, ornithine decarboxylase tests, arginine dihydrolase test, phenylpyruvic acid production tests were done. All isolates were Gram negative bacilli.

RESULTS

The results were tabulated as follows:

Serial No.	Sample Type	Number
1	ET tube-tip	28
2	Tracheal aspirate	2
3	Laryngeal suction fluid	1
Total		**31**
Table 1. Details of Number of Samples Processed		

Sl. No.	Sample Type	Total No.	Culture Positive
1	ET tube-tip	28	22
2	Tracheal aspirate	2	1
3	Laryngeal suction fluid	1	1
Total		**31**	**24**
Table 2. Break-Up of Culture Positive Samples			

Sl. No.	Name of Bacteria	No. of Isolates	%
1	*Klebsiella pneumoniae*	13/24	54.17%
2	*Acinetobacter baumannii*	8/24	33.33%
3	*Proteus mirabilis*	1/24	4.17%
4	*Citrobacter freundii*	1/24	4.17%
5	*Pseudomonas aeruginosa*	1/24	4.17%
Table 3. Species of Bacteria Isolated			

Antibiotic

Organism	Mero	Pip-Tazo	AmC	Ctx	Levo/Cip	Do	Ak/Gen	C	PB	Cl
Klebsiella pneumoniae	2	None	None	None	3	2	3	3	13	13
Acinetobacter baumannii	1	None	None	None	2	1	1	2	-	8
Citrobacter freundii	1	-	-	-	-	-	-	-	-	1
Proteus mirabilis	0	None	None	None	None	1	None	None	None	None
Pseudomonas aeruginosa	1	None	None	None	None	1	None	None	4	1
Table 4. Sensitivity pattern of isolates										

Key- Mero= Meropenem, Pip-Tazo= piperacillin-tazobactam, AmC= amoxicillin-clavulanate, Ctx= ceftriaxone, Levo/ Cip= levofloxacin/ ciprofloxacin, Do= doxycycline, Ak/ Gen= amikacin/ gentamicin, C= Chloramphenicol, PB= polymyxin B, CL= Colistin.

Ward	Klebsiella	Acinetobacter	Others
PICU and NICU	10	5	3
ICU	1	0	0
Table 5a. Culture Positive Cases: Males			

Ward	Klebsiella	Acinetobacter	Others
PICU and NICU	1	1	0
ICU	1	2	0
Table 5b. Culture Positive Cases: Females			

There are some interesting findings which must be mentioned. According to Table 2, laryngeal aspirate is 100% (1 out of 1) culture positive, tracheal aspirate is 50% (1 out of 2) culture positive and ET tube tips 79% (22 out of 28) culture positive. According to Table 3, *Klebsiella pneumoniae* is isolated in largest number. This data corroborates previous data as seen in a study by Dipti Chandra et al in 2017.

Next comes *Acinetobacter baumannii* followed by *Proteus mirabilis*, *Citrobacter spp.* and *Pseudomonas aeruginosa*. Remarkably, Gram positive organisms have not been isolated from these specimens in our study.

Table No. 5 shows that PICU and NICU provide a niche for *Klebsiella pneumoniae* and *Acinetobacter baumannii*. One reason for that may be because the patients in NICU and PICU are extremely vulnerable because of their age and correspondingly low immune status. The incidence ratio of Klebsiella and Acinetobacter in male and female is not significant in this study.

DISCUSSION

To conclude this study, we delved into a few well-known reference books to find out WHY certain organisms were being repeatedly isolated from endotracheal tube tips and aspirates all over India and abroad. No one particular reason was found, but the logical conclusions we could draw from our researches were as follows[8,9,10]:

1. *Klebsiella Pneumoniae*: Capsule, klebocin, heat-stable toxin, lipopolysaccharide and adhesion mechanisms were all responsible for making this organism practically invincible. The adhesion mechanisms were both fimbrial i.e. mannose sensitive, haemagglutination type I, type II and KPF 28. Non-fimbrial adhesion factors were CF29K and CS31A, which are plasmid encoded. The function of enterobactin and plasmid encoded aerobactin are still being researched.
2. *Acinetobacter baumannii*: This organism is well known for its virulence. The important factors responsible are adhesions, invasiveness, cytotoxicity through mitochondrial damage, lipopolysaccharide and presence of siderophores. *Citrobacter spp*. possess adhesion mechanism with mannose sensitive type I fimbriae.
3. *Proteus mirabilis:* Damages epithelium by producing urea, stimulates chemotaxis, causes calcium dependent and independent haemolysis, produces IgA ase and protease.
4. *Pseudomonas aeruginosa:* Produces chemotactic motility, colonisation due to the presence of pili and polar flagella. This bacterium is notorious for toxin mediated immune evasion. Non-diffusible exotoxin S, U, T2Y injected via type III secretary system helps to evade phagocytosis. Diffusible exotoxin, protease, phospholipase, haemolysin, elastase and pyocyanin, through the type III protein synthesis process. For this bacterium the host inflammatory response is mediated by TLR 4 and TLR 5. Pigments and a wide range of temperature tolerated by this organism also helps in pathogenicity.

All the organisms mentioned above are excellent Biofilm[11,12] producers. The mechanism may be different, but the net effect is the same. *Acinetobacter baumannii* secretes BAP or biofilm associated protein, which is regulated by BFM RS. A 2D proteomic analysis of pellicle forming *Acinetobacter baumannii* shows over-expression of Car-O which is an Opr-D homolog, helping biofilm formation. K1 capsular polysaccharide inhibits phagocytosis. Omp38 protein causes apoptosis of host cells. In case of *Klebsiella pneumoniae,* biofilm formation on abiotic surfaces is facilitated by MrkA type III fimbria and biofilm formation on human cells needs MrkD type III fimbria. TreC and SugE genes affect biofilm modulating CPS production.

Polymicrobial biofilm formation is also seen with combination of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and *Proteus mirabilis*.

Last but not the least is drug resistance[13,14] seen in all these organisms individually and more pronounced in the Biofilm community. All these factors are responsible for the huge percentage of infections in the ET tubes.

CONCLUSION

Our study results show that none of the infecting organisms were Gram positive. All our isolates were Gram negative bacilli. *Klebsiella pneumoniae, Proteus mirabilis, Citrobacter spp.* belong to the family enterobacteriaceae and *Pseudomonas aeruginosa and Acinetobacter baumannii* belong to the non-fermenters. All these organisms are highly equipped to form Biofilms on abiotic surfaces as mentioned above. Their virulence factors are also quite formidable. This study aims to point out and prime the medical fraternity to remain aware of this problem and to start appropriate management as soon as infection is suspected, keeping in mind the Gram reaction and drug resistance pattern of the bacteria commonly isolated.

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