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Prevalence of Nonfermenters in Pyogenic Infections with Antimicrobial Profile

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The non-fermentative Gram negative bacilli (NFGNB) are a group of aerobic, non-spore forming bacilli that either do not use carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation. The present study was planned to identify the non-fermenters isolated from pyogenic infections and to know their antimicrobial sensitivity pattern. A total numbers of 300 pus samples were collected from the cases of pyogenic infection, in Silchar Medical College & Hospital during the period from June 2013 to May 2014. The nonfermenters were identified by standard conventional laboratory procedure and oxidation-fermentation etc. Antimicrobial sensitivity pattern was determined by Kirby-Bauer disc diffusion method. Out of 300 pus samples, 56 (18.7%) were isolated as nonfermenters. The most common species identified was *Pseudomonas aeruginosa* 40 (71.4%) followed by *Acinetobacter baumannii* 10 (17.9%), *Pseudomonas fluorescens* 5 (8.9%) and *Pseudomonas putida* 1(1.8%). The in vitro antimicrobial sensitivity test showed 100% sensitivity by Polymyxin-B against nonfermenters isolated in the present study followed by Imipenem (82.1%), whereas Levofloxacin showed least sensitivity (33.9%). Nonfermenters are fairly common organisms associated with pyogenic infections in this region as observed in the present study. The most common isolates were *Pseudomonas aeruginosa* followed by *Acinetobacter baumannii*. The most effective antibiotics were Polymyxin-B, followed by Imipenem.

Key words: Nonfermenters, Gram negative bacilli, Pyogenic infection, Imipenem.

The non-fermentative Gram negative bacilli (NFGNB) are a group of aerobic, non-spore forming bacilli that either do not use carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation. They constitute a heterogeneous group of bacteria as they do not fall into a well defined taxonomic group.[1] The non-fermentative Gram negative bacilli share the common phenotypic features of failing to acidify the butt of Kligler iron agar (KIA) or Triple sugar iron (TSI) agar or of oxidative-fermentative media and grow significantly better under aerobic than under anaerobic condition, many strains fail to grow anaerobically.[2] They are ubiquitous in nature. Although they are commonly considered to be environmental contaminants, they have emerged as important nosocomial pathogens.[2]

Nonfermenters colonize humidifiers, ventilator machines and catheter devices in the hospital environment and they can also contaminate the dialysis fluids thereby providing opportunities for these organisms to establish infection.[2,3] Nonfermenters comprise about one fifth (or less) of all Gram negative aerobic or facultative anaerobic bacilli recovered from clinical or extra-intestinal specimens in a routine clinical microbiology laboratory.[4] Moreover, the antibiogram pattern of nonfermenters usually show multidrug resistance.[5]

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This type of study has been carried out for the first time in this part of Assam to isolate and identify the common pathogenic nonfermenters and to determine the antibiotic susceptibility pattern.

MATERIALS AND METHODS

In the present study a total numbers of 300 pus samples were collected from the cases of pyogenic infection attending the outpatient department (OPD) and admitted in the indoor ward of Surgery, ENT, Orthopedics and Obstetrics and Gynecology (O&G) department, Silchar Medical College & Hospital during the period from June 2013 to May 2014. Under strict aseptic condition pus samples were collected in the form of swabs in sterile test tubes. The samples collected were immediately transferred to Bacteriology section of Department of Microbiology for processing. The media and reagents are purchased from HIMEDIA laboratories. Mumbai, India. First, samples were inoculated in 5% sheep Blood agar and MacConkey agar culture media, then it was subjected to Gram staining of direct smear and Gram stain was examined for the presence of pus cells and any bacteria. The inoculated media were incubated aerobically at 37°C for 24 hours and incubation extended upto 48 hours, if there was no growth it was considered sterile. All the non-lactose fermenting organisms growing on MacConkey agar and those growing only on Blood agar were subjected to the Colony character, Gram staining and motility. All Gram negative bacilli or coccobacilli, oxidase positive or negative, motile or non motile, grown on MacConkey agar and Blood agar were processed further. Media used for identification of nonfermenters was Triple sugar iron (TSI) agar to determine whether the organism is able to metabolize carbohydrates or not. The organisms producing alkaline slant/no change in the butt or alkaline slant/alkaline butt in the TSI were considered as nonfermenters.

The identified nonfermenters were inoculated into oxidative-fermentative media (OF media) to differentiate between glucose oxidizer and nonoxidizer. On the basis of growth on McConkey agar media, oxidase test and oxidative-fermentative glucose tests the strains were grouped according to Weaver-Hollis scheme.[6,7] Grouped nonfermenters were further processed for species identification on the basis of motility, catalase production, nitrate reduction, arginine dihydrolase production, lysine and ornithine decarboxylase production, oxidative/fermentative utilization of mannitol, lactose, sucrose, maltose and xylose, indole production, gelatin liquefaction, hydrogen sulphide production, urease production, citrate utilization, growth at 42°C and pigment production.

Antimicrobial susceptibility of isolated nonfermenters were tested by Kirby-Bauer disc diffusion method as per the recommendation of Clinical and Laboratory Standard Institute (CLSI). The antimicrobial discs used were from HiMedia Lab Ltd. Inhibition zones were measured and reported as sensitive or resistant according to manufacturer's literature. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains. Antimicrobial discs were used for determination of sensitivity by disc diffusion test were Imipenem (IPM)-10mcg, Meropenem (MRP)-10mcg, Aztreonam (AT)-30mcg, Piperacillin/tazobactam (PIT)-100/10mcg, Ceftazidime (CAZ)-30mcg, Cefotaxime (CTX)-30mcg, Polymyxin-B (PB)-300 units, Amikacin (AK)-30mcg, Gentamicin (GEN)-10mcg, Tobramycin (TOB)-10 mcg, Ciprofloxacin (CIP)-5 mcg and Levofloxacin (LE)-5 mcg.

RESULTS

A total number of 300 cases of pyogenic infection were included in this study. Out of 300 pus samples collected, 203 were culture positive and 97 were sterile. Nonfermenters were isolated from 56 samples (18.7%).

Table 1. Preliminary grouping of Nonfermenters

Group	Oxidation of glucose	Growth on MacConkey agar	Oxidase production	No. of isolates	%
I	+	+	+	46	82.1
II	+	+	-	10	17.9

Out of total 56 nonfermenters isolated 39 (69.6%) were from hospitalized patients and 17 (30.4%) were from out-patients. Fig 2 shows distribution of nonfermenters in hospital patients. Out of 56 nonfermenters, 45 (80.4%) were isolated as pure culture and 11(19.6%) yielded mixed isolates of two organisms. Fig 3 shows the pattern of pure culture and mixed infection.

The age of the patients in this study varied from 1 year to 85 years. Most of the nonfermenters were isolated from cases above 20 years of age. The highest numbers of 17 (30.4%) cases were in the age group of 21 – 30 years, followed by 15 (26.8%) cases in the age group of above 40 years. There were 11(19.6%) cases in the age group of 11- 20 years and 8 (14.3%) cases in the age group of 31 – 40 years. Minimun number of 5 (8.9%) cases was found in the age group of 0-10 years. Nonfermenters were isolated from 38 males and 18 females, showing it to be higher in male.

Isolates of nonfermenters can be divided into 8 (eight) groups on the basis of Weaver-Hollis scheme. The grouping was done by testing the isolated strains for growth on MacConkey agar, oxidase and oxidative-fermentative glucose tests. However, in this study only 2 (two) groups of organisms were obtained and those were grouped as I and II. Majority of the isolates, 46 (82.1%) were in group I, where as group II had 10 (17.9%) isolates.

In group I, 46 species of Pseudomonas were identified in this study. *Pseudomonas aeruginosa* was the commonest isolates, 40 (71.4%) and 5 (8.9%) strains were Pseudomonas fluorescens and 1 (1.8%) strain was Pseudomonas putida. In group II, Acinetobacter baumannii 10 (17.9%) So, among all the nonfermenters isolated commonest species was *Pseudomonas aeruginosa* (71.4%) followed by Acinetobacter baumannii (17.9%).

In group I nonfermenters, out of 40 *Pseudomonas aeruginosa* isolated, 34 (85%) were pigmented and 6 (15%) were non pigmented.

Antimicrobial sensitivity of nonfermenters isolated from pyogenic infections

Antimicrobial sensitivity pattern of nonfermenters isolated in this study showed multidrug resistance. Polymyxin-B had shown maximum sensitivity (100%) followed by Imipenam (82.1%), Amikacin (80.4%), Piperacillin/tazobactam (71.4%), Meropenam (69.6%) and Ciprofloxacin (64.3%). Among other antibiotics, Aztreonam (58.9%), Tobramycin (58.9%), Gentamicin (55.4%) and Ceftazidime (46.4%) were sensitive. Cefotaxime

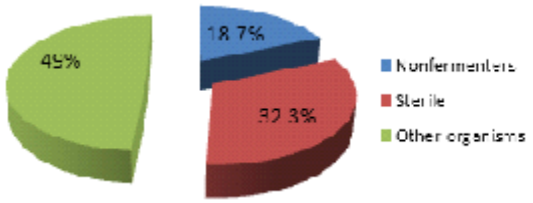

Fig. 1. Culture results of pyogenic infection

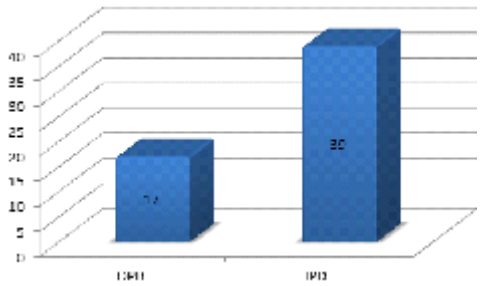

Fig. 2. Distribution of Nonfermenters in hospital patients

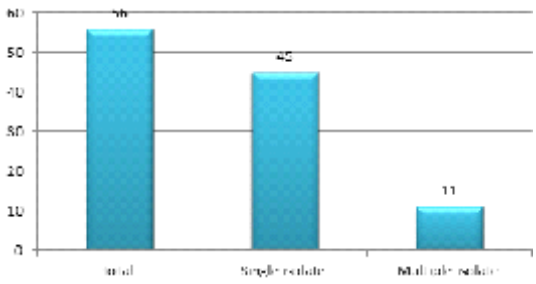

Fig. 3. Pattern of growth in nonfermenter infection

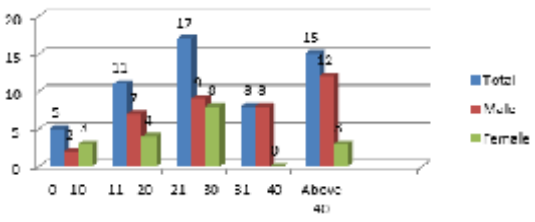

Fig. 4. Age wise distribution of 56 cases

(39.3%) and Levofloxacin (33.9%) were less sensitive. Out of 40 *P. aeruginosa* isolated in culture, 40 (100%) were sensitive to Polymyxin-B, followed by 34 (85%) were sensitive to Imipenem, 33 (82.5%) isolates were sensitive to Amikacin, 31 (77.5%) to Piperacillin/tazobactam, 29 (72.5%) to Meropenem, 27 (67.5%) to Tobramycin, 25 (62.5%) to Ciprofloxacin and 23 (57.5%) to Aztreonam.

Out of 10 Acinetobacter baumannii isolated in this study, 10 (100%) were sensitive to Polymyxin-B, 8 (80%) were sensitive to Amikacin followed by 7 (70%) were sensitive to Imipenem, 6 (60%) to Meropenem, Aztreonam, Ciprofloxacin and Gentamicin. Polymyxin-B, Imipenem, Piperacillin/ tazobactam, Ciprofloxacin, Meropenam, Aztreonam, Ceftazidime, Amikacin were effective against both Pseudomonas fluorescens and Pseudomonas putida species. Pseudomonas putida was resistant to Tobramycin and Levofloxacin

DISCUSSION

Nonfermenters are a group of aerobic nonsporing Gram negative bacilli found primarily free in nature and as commensals whose pathogenic potentials are well established.[8] Previously non-fermentative Gram negative bacilli were considered to be non pathogenic and of very little significance. Recently, rate of infection by non-fermentative Gram negative bacilli is rising, especially in hospitalized and immune-compromised patients.

In the present study 18.7% of nonfermenters were isolated from 300 pus samples. Almost similar observation made by Bose S *et al*.[9], 2013 who isolated 26.25% nonfermenters from pus samples. A very high prevalence of nonfermenters in pus was observed in the study done by Patel PH *et al*.[10], 2013 who isolated 58.65% of nonfermenters. A lower prevalence of nonfermenters was observed in the study done by Kaushal ML *et al*.[11] , 1996 who obtained 8.36% of nonfermenters from 2,140 pus samples. In most other studies prevalence of nonfermenters in pus samples were not mentioned (Mishra B *et al*.,[12] 1986, Yashodhara P *et al*.[13], 1997, Veenu *et al*.[14], 1998, Kharangate NV *et al*[8]., 2001, and Vijaya D *et al*.[15], 2000).

In this study, out of 56 nonfermenters isolated 80.4% formed single bacterial isolate while 19.6% formed mixed isolate of two organisms. Similar observation was made by Kaushal ML *et al*[11]., 1996 who isolated 83.2% as pure and 16.8% as mixed culture. Mishra B *et al*[12]., 1986 isolated 85.5% as pure and 14.5% as predominat organism, while isolating nonfermenters from clinical samples.

In the present study, out of 56 nonfermenters isolated from different pus samples, *P. aeruginosa* was the most common constituted 71.4%. Similar observation was formed by other studies done by Veenu *et al*.[14], 1998 who found 73.4%, Yashodhara P *et al*[13].,1997 who found 64%, Kaushal ML *et al*.[11], 1996 who found 88.8%.

Table 2. Species identification of 56 Nonfermenters

Group	Species	No. of isolates	%
I	Pseudomonas aeruginosa	40	71.4
	Pseudomonas fluorescens	5	8.9
	Pseudomonas putida	1	1.8
II	Acinetobacter baumannii	10	17.9

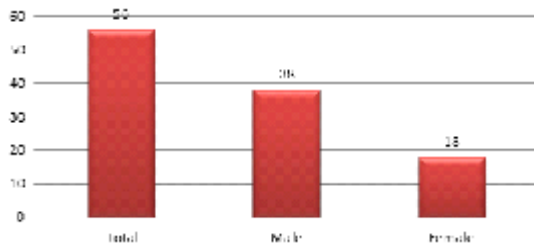

Fig. 5. Sex wise distribution of 56 cases

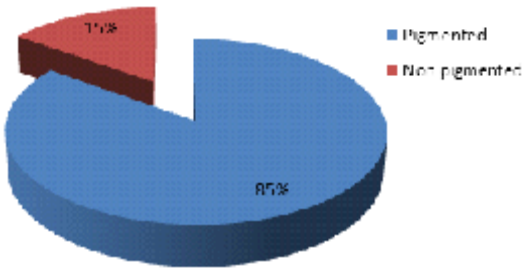

Fig. 6. Distribution of *Pseudomonas aeruginosa* on the basis of pigmentation

In the present study, Polymyxin-B was found to be the most effective drug which showed 100% sensitivity to all the isolates. Similar observation was made by the studies done by Bose S *et al*., 2013 and Thipperudraswamy T *et al*.[16], 2014 showed 100% sensitivity to all isolated nonfermenters, Biglari S *et al*[17]., 2013 showed 100% sensitivity to Acinetobacter species, Rashid H *et al*[18]., 2014 and Pathi B *et al*[19] showed 100% sensitivity to *P. aeruginosa*.

CONCLUSION

Nonfermenters are fairly common organisms associated with pyogenic infections in this region as observed in the present study. The most common isolates were *P. aeruginosa* followed by *A. baumannii*. The most effective antibiotics were Polymyxin-B, followed by Imipenem, Amikacin and Piperacillin/tazobactam. Organisms are resistant to drugs commonly employed in therapy emphasize that nonfermenters need to be taken more seriously and should not be discarded as mere contaminants or non pathogens. The sensitivity pattern changes from hospital to hospital and population to population. Minimized use of available antimicrobial, regular antimicrobial susceptibility surveillance and strict infection control measures are required to control this emerging antibiotic resistance among nonfermenters.

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