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PREVALENCE OF ESBL PRODUCING OXIDASE POSITIVE NON FERMENTING GRAM NEGATIVE BACTERIA ISOLATED FROM VARIOUS CLINICAL SPECIMENS AT A TERTIARY CARE HOSPITAL IN CHENNAI.

Microbiology

KEYWORDS: ESBL, NFGNB, Drug Resistance.

Karthiga L — Assistant Professor, Department of Microbiology, ACS Medical College & Hospital, Chennai. *Corresponding Author

Srivani Ramesh — Assistant Professor, Department of Microbiology, Dr.ALM PGIBMS, University of Madras, Chennai.

ABSTRACT

Oxidase positive nonfermenting bacteria (NFGNB) have emerged as potential nosocomial pathogens and are becoming resistant to commonly used antimicrobial agents, therefore the present study was under taken to investigate the prevalence of Extended Spectrum Beta Lactamases (ESBLs) production in various species of oxidase positive Non Fermenting Gram Negative Bacteria (NFGNB). Isolates of oxidase positive NFGNB from various clinical specimens were obtained from Department of Microbiology, Madras Medical College & Hospital, Chennai and speciated using conventional biochemical methods and antibiotic susceptibility testing was done as per CLSI guidelines. ESBL production was detected by phenotypic confirmatory disc diffusion test (PCT). ESBL phenotypes were subjected bla TEM screening using PCR. Out of 40 isolates from various clinical samples, Pseudomonas aeruginosa was the commonest accounting for 70% followed by Pseudomonas fluorescens 15%. Among the 40 isolates, 35% were multidrug resistant, 27.5% of the isolates were found to be ESBL producers. ESBL producers were negative for bla TEM gene by PCR assay. To conclude significant percentage of NFGNB were found to be ESBL producers in our study. To know the actual incidence of ESBLs in these organisms, all the NFGNB should be speciated and screened for ESBL production to avoid the spread of drug resistance.

INTRODUCTION

Oxidase positive nonfermentative gram-negative bacteria (NFGNB) are generally thought to be harmless environmental saprophytes and only *Pseudomonas aeruginosa* is clinically thought to be important. Various species of oxidase-positive NFGNB are nowadays gaining importance as nosocomial pathogens and infections with them are increasingly being reported from hospitalized patients due to heightened awareness (Kalawat *et al.*,2012). NFGNB account for approximately 15% of all gram negative bacilli cultured from clinical samples (Siou *et al.*,2009). Non-fermenting gram negative bacilli are intrinsically resistant to many antibiotics and are known to produce extended spectrum beta lactamases and metallo beta lactamases (Malini *et al.*,2009). Extended spectrum beta lactamases (ESBLs) represent a major group of beta lactamases responsible for resistance to oxyimino-cephalosporins and aztreonam and currently being identified in large numbers throughout the world. ESBLs are encoded by both chromosomal and plasmid genes. Hence, acquired resistance to beta lactams can result in therapeutic failure, particularly when it is associated with resistance to other classes of drugs, such as aminoglycosides and fluoroquinolones (*Hakemi Vala M et al.*,2013). With this background, the present study aimed to identify the prevalence of various species of oxidase positive non fermenting bacteria from different clinical specimens and to screen them for ESBLs production.

MATERIALS AND METHODS

Oxidase positive NFGNB isolates from various clinical samples were collected from the Department of Microbiology, Madras Medical College & Hospital, Chennai and the study was conducted at the Department of Microbiology, Institute of Basic Medical Sciences, University Of Madras, Chennai. The isolates were speciated using conventional biochemical tests (Koneman *et al.*,2004). Antibiotic susceptibility testing was done as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Isolates were screened for the ESBL production by combined disc method (single disc synergy method) using ceftazidime 30g disc alone and in combination with clavulanic acid (ceftazidime 30g + clavulanic acid 10g) as per CLSI recommendations. Isolates showing more than 5mm increase of zone size in ceftazidime + clavulanic acid combination disc were considered as ESBL producers. All the ESBL positive isolates were tested genotypically for the presence of TEM type of ESBL using PCR analysis (*Xiaofei Jiang et al.*, 2006).

RESULTS

Majority of the isolates were from respiratory specimens followed by pus (Fig.1). Out of 40 isolates of oxidase positive NFGNB, 70% were *Pseudomonas aeruginosa*, 15% were *Pseudomonas fluorescens*, 7.5% were *Pseudomonas putida*. In the present study, we have isolated 5% isolates of *Brevundimonas dimunita* & 2.5% of *Burkholderia cepacia* complex (Fig 2).

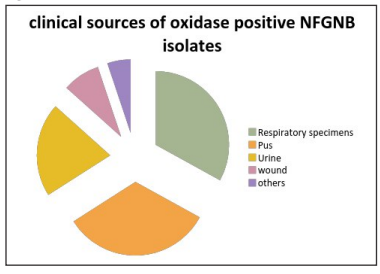

Fig. 1

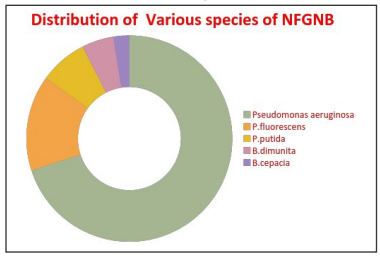

Fig. 2

Antibiotic sensitivity done with a panel of antibiotics showed a maximum sensitivity to imipenem (96.5%), ciprofloxacin (65%), tobramycin (50%). A higher resistance rate of 75% was seen for gentamicin and cefepime, 42% of NFGNB isolates were resistant to cefotaxime and 21% were resistant to ceftazidime. Among the 40 isolates, 14 were found to be multidrug resistant, where multi drug resistance was higher in *P.aeruginosa* (64%) followed by *P.putida* (14.28%) and *B.dimunita* (14.28%), *B.cepacia* complex (7.14%), 11 out 40 isolates were ESBL positive by combined disc method. *P.aeruginosa* (91%) showed maximum ESBL prevalence followed by *B.cepacia* complex (9%), 35% of the ESBL producers were found to be multi drug resistant. The 28 isolates of *P.aeruginosa*, 10 isolates

were ESBL producers. All the 11 ESBL positive isolates were negative for *bla* TEM type ESBL by PCR analysis.

DISCUSSION

Many innocuous species of oxidase positive non fermenting gram negative bacteria has been emerging as potential nosocomial pathogens. Empirical use of third generation cephalosporins in such hospital settings has favored the spread of ESBLs in these organisms (Shah *et al.*,2003). Present study revealed the highest incidence of *P.aeruginosa* among the NFGNB, which was similar to the study conducted by Martino *et al.*, 2000 where *P.aeruginosa* accounted for a higher percentage (35%) among the NFGNB. Isolation rate of *P.putida* in our study was 7.5% which is more or less similar to the finding of Martino *et al.*, 2000, where it was 10%. The percentage of isolation was too small for *B.dimunita*, however analyzing more number of samples would confirm their isolation rate. Only one isolate of *B.cepacia* complex was obtained in our study but Martino *et al.*, 2000 had shown a higher incidence of (14%) in their study cohort which involves bacteraemia caused by NFGNB in immunosuppressive patients. In the present study, majority of the *P.aeruginosa* strains were from pus. Similar findings obtained in a study conducted by Chaudhuary *et al.*,2003. Majority of ESBL producers were obtained from pus in our study. About 14/40 (35%) isolates were found to be MDR in our study, out of which 9(63%) isolates were *P.aeruginosa*. Taneja *et al.*,2008, reported 28.5% of MDR *P.aeruginosa* in their study. Multidrug resistance was also observed in 2/3 isolates of *P.putida,* 2/2 isolates of *B.dimunita* & 1/1 isolate of *B.cepacia* complex. It is noteworthy that though the number was small, they were found to be MDR. About 11/40 (27.5%) NFGNB isolates were ESBL positive by combined disc method, which is more or less similar to the finding of Rodrigues *et al*, 2004, where a prevalence of 28.5% among the NFGNB was reported. Out of 11 ESBL positive isolates, 10 were *P.aeruginosa*. There is a similar finding in the study conducted by Taneja *et al*,2008, where 27/43 (27.9%) *P.aeruginosa* isolates were ESBL positive. Whereas Lata *et al.*,2004 have reported the absence of ESBL in *P.aeruginosa* in their study from north india which involved double disc synergy test for ESBL screening. Among the ESBL positive isolates, 27% were resistant to ciprofloxacin in the present study. It is a comparable percentage to the previous study done by Amita Jain *et al.*,2003, where the prevalence was 26%. Only 35% of the ESBL producers are MDR in our study which is similar to the finding of Taneja *et al.*,2008, where only (36.5%) of the ESBL positive strains are MDR. MDR in non ESBL producers may be due to other beta lactamases like Amp C.

All the 11 ESBL positive isolates analysed by PCR were not positive for *bla* TEM type of ESBL gene. Hence, the present study reveals that ESBLs other than TEM type may be responsible for the ESBL positivity of the isolates.

SUMMARY & CONCLUSION

From the present study, it may be concluded that, significant percentage of NFGNB isolates were ESBL positive. The real picture of ESBL incidence among oxidase positive NFGNB could not be retrieved due many factors including lack of CLSI guidelines, many laboratories considering most of the NFGNB as contaminants and do not emphasize on ESBL screening. Under reporting of ESBLs may allow the dissemination of ESBL producing strain and can cause serious outbreaks particularly in nosocomial settings. Hence, routine screening for ESBLs is mandatory as this may help in regulating antibiotic policy which promotes the judicial use of antibiotics and thereby preventing the emergence of drug resistant strains.

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