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Microbiology

KEY WORDS: ESBL (Extended spectrum β lactamase), MBL(Metallo-β-lactamses), Amp-C β lactamases, MDR (Multi drug resistance).

STUDY OF BACTERIOLOGICAL PROFILE OF LACTOSE FERMENTING AND NON-LACTOSE FERMENTING BACTERIA FROM TERTIARY CARE HOSPITAL WITH SPECIAL REFERENCE TO EXTENDED SPECTRUM BETA LACTAMASE (ESBL), METALLO-BETA- LACTAMASES (MBL) AND AMP-C PRODUCTION.

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ABSTRACT

Objective: Purpose of study was to evaluate bacteriological profile of gram negative bacteria from various clinical samples, received in Microbiology Department at tertiary care teaching hospital in Solapur and to analyze various types of drug resistance seen in gram negative bacteria like ESBL, MBL and Amp-C production in MDR strains. **Material & Method:** Various clinical samples received in Microbiology department were inoculated on culture media and incubated overnight, growth was noted next day, later conventional methods were used for identification of gram negative bacteria. Antibiotic susceptibility testing was done, organisms resistant to three or more drugs were thereafter subjected to various phenotypic tests for identification of various drug resistance. **RESULTS:** Out of 1566 samples, 512(32.7%) samples were sterile. Growth was seen in 1054 (67.30%) samples. Out of which 854 (54.53%) were lactose fermenter gram negative bacteria (LFGNB) while 200 (12.77%) were non lactose fermenter gram negative bacteria (NFGNB). Among LFGNB and NFGNB maximum sensitivity was seen for imipenem 75.04% followed by amikacin 69%, piperacillin tazobactam 65%.Cefotaxim 60% Maximum resistance was noted for fluroquinolones 91.42% and cotrimoxazole 85%.Among LFGNB ESBL producer were 290 (34.0%). MBL producer were 218 (25.50%). Amp-C producer were 22 (2.57%).Among NFGNB ESBL producers were 44 (22.0%) . Imipenem resistant NFGNB isolates were 54 of which MBL producers were 18(33.33%) and Amp-C producers were 29 (14.5%).

INTRODUCTION

Throughout the world bacterial infections are leading cause of morbidity and mortality, which is responsible for increased health care cost and accounts for major burden on patients and public health system of any country.1,2. Patients admitted to hospital for prolong periods are often colonized by ESKAPE group of bacteria which are commonly present in hospital environment and are usually Multi drug resistant (MDR) strain. They are usually plasmid coded so they may rapidly spread from one strain to another. The increased risk of bacterial infection is further compounded by rising trends of antibiotic resistance in commonly implicated organism all over the world3. Multi drug resistance (MDR) to commonly used antibiotics among clinically important gram negative pathogens is on rise4 which has led to increased concern among physician due to limited options left for treatment of such cases. The production of extended spectrum β lactamase (ESBL) or Amp-C type β lactamase or Metallo β lactamase (MBL) is often associated with resistance to aminoglycosides and fluoroquinolones5. Antibiotic susceptibility data of clinical isolates is of immense value to rational selection of antimicrobial agents and for development of appropriate antibiotic policy.

Therefore present study was undertaken to identify bacteriological profile from various clinical samples with special reference to different antibiotic resistance like ESBL,MBL and Amp-C type of lactamase.

MATERIAL & METHOD: The study was conducted between Dec 2015 to July 2017. Various clinical samples received in Microbiology department were inoculated on blood, Mac conkey agar and CLED media (in case of urine sample) after overnight incubation growth was noted and conventional methods such as biochemical test were used for identification of gram negative bacteria. Antibiotic susceptibility testing was done using Kirby Bauer disk diffusion method, organisms resistant to three or more drugs or resistant to all Penicillins, Cephalosporins all three generations, Monobactems, and or Cephamycin and Carbapenems were thereafter subjected to various phenotypic tests for identification of various types of drug resistance like ESBL, Amp-C and MBL production as per CLSI guidelines6.

RESULTS

Out of 1566 samples, 512(32.7%) samples were sterile. Growth was seen in 1054 (67.30%) samples.

Maximum samples were reported from IPD patients. Males 630 (59.77%) were commonly infected by infections caused by gram negative bacteria as compared to females 424 (40.22%).

Out of 1054 samples with growth 854 (54.53%) were lactose fermenter gram negative bacteria (LFGNB) while 200 (12.77%) were non lactose fermenter gram negative bacteria(NFGNB). Majority of LFGNB isolates were E. coli 482 (45.73%), followed by Klebsiella pneumonia 262(24.90%) and Enterobacter species 110 (12.0%). In non-fermenter gram negative bacilli majority of NFGNB were Pseudomonas aeruginosa 112 (56%) followed by Acinetobacter baumanii 71(35.5%), Pseudomonas fluorescens 7 (3.5%), Acinetobacter lwofii 6 (3%), Burkholderia cepacia complex 2 (1%), Sphingomonas paucimobilis 2 (1%).

Among lactose fermenters bacteria 854 (54.53%), majority were reported from urine 296 (34.66%), followed by blood 267 (31.26%), pus 217 (25.40%), sputum 65 (7.61%), endotracheal aspirate 6 (0.7%) and pleural fluid 3 (0.35%).

However among non-fermenter gram negative bacteria majority of NFGNB were isolated from pus 95 (47.5%) followed by sputum 55 (27.50%), urine 23 (11.5%) blood 17 (8.5%), endotracheal aspirate 07 (3.5%) and pleural fluid 3 (1.5%).

Among LFGNB and NFGNB maximum sensitivity was seen for Imipenem 75.04% followed by Amikacin 69%, Piperacillin tazobactam 65%.Cefotaxim 60%, Nitrofurantoin (for urine samples only)58% Maximum resistance was noted for Fluroquinolones 91.42% and Cotrimoxazole 85%. Among lactose fermenter gram negative bacteria (n=854), ESBL producers were 290 (34.0%) Maximum ESBL producer were seen in E.coli 227 (27.0%) followed by Klebsiella pneumonia 63 (7.4%).

In LFGNB MBL producers were 218 (25.52%). Highest MBL producer among lactose fermenter gram negative bacteria were seen in E.coli 123(14.40%) followed by Klebsiella pneumonia 95 (11.12%) Maximum Amp-c producers among LFGNB were 22 (2.57%) of which E.coli were 15 (2.0%), followed by Klebsiella pneumoniae 07 (0.81%) were Amp-C producers. (as shown in table-1).

Among NFGNB ESBL production was seen in 44 (22.0%). Maximum ESBL producers were in Pseudomonas aeruginosa 36 (32.14%) followed by Acinetobacter baumanii 8 (11.30%) . Among NFGNB 54 isolates were imipenem resistant of which 18 (33.33%) were MBL producers. Amp-C production was seen in 29 (14.50%) NFGNB isolates (as shown in table-1)

TABLE-1 showing distribution of various antibiotic resistance in Gram negative bacteria

GROUP	ESBL	MBL	Amp-C
LFGNB*	290 (34.0%)	218 (25.52%)	22 (2.57%)
NFGNB**	44 (22.0%)	18 (33.33%)***	29 (14.50%)

LFGNB* (lactose fermenter gram negative bacteria), NFGNB (Non-fermenter gram negative bacteria)**
***** only 54 NFGNB isolates were resistant to Imipenem so only these were checked for MBL production. Of which only 18 (33.33%) were MBL producer.**

DISCUSSION

In the present study LFGNB were predominant 854 (54.53%), compared to NFGNB 200 (12.77%). Similar results were seen by Rachna bohra et al (2017) & Narinder kaur et al (2017). Maximum samples were reported from IPD patients similar results were seen by study done by T Sering et al (2008) & Deshmukh D.G. et al (2011). In the present study males 630 (59.77%) were commonly infected by infections caused by gram negative bacteria as compared to females 424 (40.22%) similar finding were seen in Narinder Kaur et al (2017) & Kamble D et al (2015).

Majority of LFGNB isolates were E. coli 482 (45.73%), followed by Klebsiella pneumonia 262(24.90%) and Enterobacter species 110 (12.0%). In non-fermenter gram negative bacilli majority of NFGNB were Pseudomonas aeruginosa 112 (56%) followed by Acinetobacter baumanii 71(35.5%) similar results were reported by Rachna Bohra et al (2017) & Kamble D et al (2015).

In the present study lactose fermenter bacteria 854 (54.53%), majority of LFGNB were reported from urine 296 (34.66%), followed by blood 267 (31.26%), pus 217 (25.40%), sputum 65 (7.61%), endotracheal aspirate 6 (0.7%) and pleural fluid 3 (0.35%) similar results were seen by study done by T Sering et al (2008) & Kamble D et al (2015). However among non-fermenter gram negative bacteria majority of NFGNB were isolated from pus 95 (47.5%) followed by sputum 55 (27.50%), urine 23 (11.5%) blood 17 (8.5%), endotracheal aspirate 07 (3.5%) and pleural fluid 3 (1.5%). In the study done by kotgire Santosh A et al (2016) LFGNB & NFGNB were most commonly isolated from urine sample(46.01%) followed by pus sample(29.43%,) blood (12.48%), other samples (6.04%). In study done by Rachna Bohra et al (2017) LFGNB and NFGNB were most commonly isolated from urine (45.51%), followed by blood (24.76%), pus (15.09%), ET tube secretion (8.72%), sputum (3.77%) and BAL (2.12%). Present study shows similar results in case of LFGNB where majority of LFGNB were isolated from urine followed blood and pus sample. However in case of NFGNB our results are in contrast to the findings of Kotgire Santosh A et al (2016) and Rachna Bohra et al (2017) where NFGNB most commonly isolated from urine sample followed by blood and pus, this may be due to difference in study population, environmental conditions and other contributing factors.

Among LFGNB and NFGNB maximum sensitivity was seen for Imipenem 75.04% followed by Amikacin 69%, Piperacillin tazobactam 65%.Cefotaxim 60%. Maximum resistance was noted for Fluroquinolones 91.42% and Cotrimoxazole 85%.Among lactose fermenter gram negative bacteria (n=854), ESBL producers were 290 (34.0%) .Maximum ESBL producer were seen in E.coli 227 (27.0%) followed by Klebsiella pneumonia 63 (7.4%). In the study done by Kotgiri Santosh A et al (2016) ESBL detection among LFGNB was 18.37%, which is less than present study. However study done by Rachna bohra et al (2017) high rate of ESBL was reported 65.07%. Highest MBL producer among lactose fermenter gram negative bacteria were seen in E.coli 123(14.40%) followed by Klebsiella pneumonia 95 (11.12%) Maximum Amp-c producers among LFGNB were 22 (2.57%) of which E.coli were 15 (2.0%), followed by Klebsiella pneumoniae 07 (0.81%) were Amp-C producers. (as shown in table-1). Various studies have shown variation in MBL and AmpC beta lactamase type of resistance among LFGNB, overall still ESBL accounts to most common type of antibiotic resistance encountered among LFGNB as compared to MBL and Amp-C but recent studies shows there is rapid increase in MBL producer LFGNB.

However among studies done by various workers it has been noted that in case of NFGNB ESBL production is comparatively less as compared to MBL resistance, more strains are found resistant to carbapenems. In the present study NFGNB ESBL production was seen in 44 (22.0%). Maximum ESBL producers were in Pseudomonas aeruginosa 36 (32.14%) followed by Acinetobacter baumanii 8 (11.30%) . Among NFGNB 54 isolates were imipenem resistant of which 18 (33.33%) were MBL producers. Amp-C production was seen in 29 (14.50%) NFGNB isolates (as shown in table-1).Study done by Rachna Bohra et al (2017) shows about 58.9% of carbapenem resistance which is higher than the present study.

Variation in antibiotic susceptibility pattern may be attributed to difference of geographical conditions, study group, literacy level, hygiene level and socioeconomic status, as different study have difference in the above condition therefore results may show variability.

CONCLUSION

Antibiotic resistance is emerging worldwide, it is essential to prevent injudicious use of antibiotics, discourage issue of over the counter drug availability. Improve awareness among community as well as health care workers on the importance of hand washing. We must adhere strictly to hospital infection control policies, and be vigilant and report irregularities actively to the concerned authority. This study will help in formulation of hospital based antibiotic policy and implement antibiotic stewardship program in our institution.

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