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Phenotypic Screening for Asymptomatic Rectal Colonization by Resistant *Enterobacteriaceae* and Nasal MRSA Colonization in Critical Care Patients

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A B S T R A C T

Critical care populations are at high risk for Multi drug resistant organisms (MDRO) colonization because of rampant use of broad-spectrum antibiotics leading to colonization with resistant strains, Screening for MDR organisms is one of the many approaches needed to deal with the very major clinical problem concerning drug resistance. This study was planned to look for antibiotic resistance by simple phenotypic methods to detect Resistant *Enterobacteriaceae* include broad spectrum β-lactamase producing *Enterobacteriaceae* (ESBL and AmpC β-lactamases) and carbapenem-resistant *Enterobacteriaceae* (CRE)and or combinations of any in one go and nasal screening for Methicillin Resistant Staphylococcus Aureus (MRSA). Total of 110 patients admitted intensive care unit were screened by taking rectal and nasal swab over period of year. In our study 234-gram negative bacilli were isolated from 110 rectal swabs among those were 54(23.7%) ESBL, 22(9.4%) Amp C, and 30 (12.8%) isolates were carbapenem resistant which includes 21(8.9%) isolates were cabapenemase producer by MHT and MBL screen and 9(2.64%) isolates were carbapenemase +Amp C+ESBL combined producers. Nasal MRSA carriage rate was 8%. Present study emphasis the need for strong infection control programs to detect colonization of gut and other anatomical site by multidrug resistant organisms. Need to reduce overuse of antibiotics and establish good antibiotic stewardship programs and implementation of screening in intensive care unit.

Introduction

Human body commensal flora is a diverse ecosystem which is inhabited by aplethora of bacteria (Qin *et al.*, 2010). The healthy microbiota provides protective functions including ability to prevent colonization and or expansion of pathogens. Changes in the composition of the gut flora, may reduce colonization resistance and select for antibiotic resistance and which can happen silently, leading to the selection of highly resistant bacteria. An increase in gut permeability allows Bacterial translocation that is the invasion of indigenous intestinal bacteria through the gut mucosa to normally sterile tissues and the internal organs. (Vaishnavi, 2013) Endotoxins and other toxins can also cross the gut barrier. Bacteremia and endo-toxinaemia are among the mechanisms involved in severe sepsis and multiple organ failure (Arrieta *et al.*, 2006).

MDROs are defined as microorganisms, predominantly bacteria, that are resistant to one or more classes of antimicrobial agents. Critical care populations considered at high risk for Multi drug resistant organisms (MDRO) colonization based on factors such as antibiotic exposure history, presence of underlying diseases, prolonged duration of stay, exposure to other MDRO colonized patients, patients transferred from other facilities known to have a high prevalence of MDRO carriage, or having a history of recent hospital or nursing home stays so thereby induce healthcare-associated infections, undergo cross-transmission to other individuals, and cause limited outbreaks (MDROGuidelines2012 & MRGN guidelines 2016)

Staphylococcus aureus is a member of commensal micro flora and readily colonizes the anterior nares. Nasal carriage of S.aureus act as endogenous reservoir for clinical infections in colonized individuals or as a source of cross colonization for community spread (Kashmir *et al.*, 2014)

Screening for Multi-resistant Gram-negative bacilli (MRGN) organisms by a sensitive, specific and cost effective screening test may help the clinicians in the choice of appropriate antimicrobial therapy, provide baseline data about the epidemiology of MDR pathogen, to guide policy recommendations and help in infection control by early identification of patients, thereby facilitating an informed decision about infection control interventions These interventions may include notification to the concerned clinical and nursing team, infection control precautions such as isolation or cohort nursing of such patients, use of decolonization regimens, use of antibacterial prophylaxis during surgery or other invasive interventions of use of an appropriate agent for empirical antibiotic therapy in case of unconfirmed infection in a colonized patient (Bhattacharya 2011 & Deepa *et al.*, 2014). This study was planned to look for antibiotic resistance by simple phenotypic methods to detect Resistant.Multi-resistant Gram-negative bacilli include broad spectrum β-lactamase producing *Enterobacteriaceae* (ESBL and AmpC β-lactamases) and carbapenem-resistant Enterobacteriaceae (CRE)and or combinations of any in one go and nasal screening for Methicillin Resistant Staphylococcus Aureus(MRSA).

Materials and Methods

The present observational study was carried out in the Department of Microbiologyin tertiary care hospital.

Inclusion criteria

Patients admitted to intensive care unit with factors such as antibiotic exposure history, presence of underlying diseases, prolonged duration of stay, exposure to other MDRO colonized patients, or having a history of recent hospital or nursing home stays.

Exclusion criteria

Patients should not be a health worker, should not have any history of immuno-compromised status and patients who are not receiving antimicrobials.

Sample collection

2 Nasal swabs and 1 Rectal swab were collected from each patient admitted to intensive care unit.

Nasal swabs were taken by swirled while applying even pressure inside anterior nares with a sterile cotton swab (16). Rectal samples were collected in the sterile swab by inserting the swab 1cm into the rectum while rotating the swab. Rectal swabs are an appropriate

alternative to stool specimens (Lerner *et al.,* 2013). Total transit time to the laboratory was within 30 minutes. Samples were processed as per the standard protocols (Deepa *et al.,* 2014 & Lerner *et al.,* 2013)

All isolates of staphylococcus aureus from nasal swabs were processed routinely and tested with 30 mg cefoxitin discs (Hi-Media) on Mueller–Hinton agar plates by disk diffusion methodfor MRSA detection ((M100 27th edition 2017),16,17).

Resistant *Enterobacteriaceae* isolates to one or more classes of antimicrobial agents, resistant to third generation cephalosporins, resistant toeither of the carbapenems namely Imipenem, Ertapenem or Meropenem were selected for study. All the selected gram negative Enterobacteriaceae were tested by disc placement method (Deepa *et al.,* 2014, Neena *et al.,* 2012 & Shamsadh Begum *et al.,* 2015).

The lawn culture of test organismwas made on Muller–Hinton agar (MHA) as done for discdiffusion antimicrobial susceptibility test. In the center ofthe plate, imipenem (10 µg) (Inducer) disc was applied. At Antimicrobial susceptibility: the distance of 20 mm, the disc of cefotaxime (30 µg) was placed. From this disc, in a circular manner, clockwise, the discs of cefoxitin (30 µg) (Inducer), ceftriaxone (30 µg), ceftazidime (30 µg), ceftazidime + clavulinic acid (30/10µg), and aztreonam (30 µg) were placed such that any two adjacent discs were 20 mm apart from center to center (Figure 1). On overnight aerobic incubation at 37°C, the diameters of zones of inhibition were measured and interpreted as follows:

Extended-spectrum b-lactamase (Figure 2)

Zone diameter for aztreonam ≤27 mm, cefotaxime≤27 mm, ceftazidime ≤22 mm, and ceftriaxone ≤25 mm.

Susceptible to cefoxitin.

Increase in zone size with addition of inhibitor (ceftazidime+ clavulanic acid) by 5 mm or more.

AmpC b-lactamase (Figure 3)

Inducible

The blunting of zone of inhibition of Ceftazidime discs toward inducers

No increase of zone size with addition of inhibitor

Depressed mutants (DM)

Resistant to cefoxitin (≤ 14 mm) and cefotaxime

No increase of zone size with addition of inhibitor

Metalloβ-lactamases

Strains showing resistance to imipenem.

Multiple mechanisms

Resistant to cefoxitin

Blunting of zone toward inducer

Increase of zone size with addition of inhibitor by 5 mmor more.

The isolates showing resistance either of Carbapenemswere further analyzed as Carbapenem resistance in Gram-negative rods is mainly due to two mechanisms: first the production of carbapenem-hydrolyzing enzymes (i.e. serine carbapenemases and metallo-β-lactamases, and second the combination of membrane impermeability with production of ESBLs, pAmpC or ampC

overexpression (MDROGuidelines 2012). Double disc potentiation test (Figure 6) and Modified Hodge test (Figure 5) (Neena *et al.,* 2012; Shamsadh Begum *et al.,* 2015) was done on those carbapenem resistant isolates.

Modified Hodge test (Lee *et al.,* 2001 and Yong, *et al.,* 2002)

An overnight culture suspension of E. coli ATCC 25922 adjusted to 0.5 McFarland standard was inoculated using a sterile cotton swab on the surface of a MHA. After drying 10 µg imipenem disc was placed at the center of the plate and the test strain was streaked heavily from the edge of the disc to the periphery of the plate. The plate was incubated overnight at 37°C. Indentation produced in the zone of inhibition produced by the imipenem indicates a positive test. Maximum four strains can be tested at a time (all four directions) which gives a presence of a "cloverleaf shaped" zone of inhibition if all four test strains are positive for MBL production

Double disc potentiation test (Lee *et al.,* 2001 & Yong, *et al.,* 2002)

A 0.5 M EDTA solution was prepared by dissolving 186.1 g of disodium EDTA. (REACHEM, Chennai, India) in1000 ml of distilled water. The pH was adjusted to 8.0 by using NaOH (HI-MEDIA) and was sterilized by autoclaving.

An overnight liquid culture of the test isolate was adjusted to a turbidity of 0.5 McFarland standard and spread on the surface of a MHA plate. Two 10 µg imipenem discs were placed on the agar 15 mm apart (center to center). 10 µl of0.5 M EDTA is added to one of the imipenem disc to get a desired concentration of 750 µg. After incubating overnight at 37°C, the presence of an expanded growth inhibition zone between the two discs or increase of zone size of more than 7 mm in the disc potentiated with the EDTA (chelating agents) was interpreted as positive for MBL production

All the tests and their interpretations were according to the CLSIGuidelines (M100 27th edition 2017) and well-accepted methods by various authorities. (Lerner A *et al.,* 2013, Rodrigues C *et al.,* 2004, Neena V *et al.,* 2012 & Shamsadh Begum *et al.,* 2015)] the identity of the isolates and resistance pattern was further confirmed by using Vitek 2 GN ID (BioMerieux, France) with appropriate quality control.

Results and Discussion

Total of 110 patients admitted intensive care unit were screened over period of year.110 nasal swabs yielded 117 isolates most common being methicillin sensitive coagulase negative staphylococcus(MSCONS)42.7%, methicillin resistant coagulase negative staphylococcus (MRCONS) 34.18%, methicillin resistant coagulase positive staphylococcus(MRSA)8%, methicillin sensitive coagulase positive staphylococcus (MSSA) 10.25%. Nasal swab also yielded Serratia, Pseudomonas aeruginosa, Escherichia coli in five critically ill patients (Table 1).

110 rectal swabs yielded 234 isolates Resistant isolates toone or more classes of antimicrobial agents to third generation cephalosporins, either of the Carbapenems namely Imipenem, Ertapenem or Meropenem or Cefoxitin by disk diffusion method were selected for the study. Of the 234 isolates from rectal swab 112 isolates were multi drug resistant (Table 2). Among 125 (53.41%) isolates of Escherichia coli showed 68% were multi drug resistant.50 (1.47%) *Klebsiella spp.* showed 86% multi drug resistant. Only 7 of Enterobacter species were isolated but resistance rate was high as71%. There were few isolates of Citrobacter species, Proteus species.

Table.1 Isolates from nasal samples

Organisms	No of isolates.
MRCONS	40(34.18%)
MSCONS	50(42.7%)
MRSA	10(8%)
MSSA	12(10.25%)
Serratia maracescence	1(0.008%)
Pseudomonas aeruginosa.	2(0.017%)
Escherichia coli.	2(0.017%)
Total	117

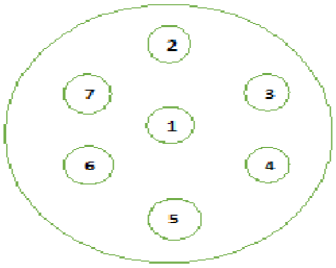
Table.2 Isolates from rectal swabs

Organisms	No of isolates.	MDRO
Escherichia coli	125 (53.41%)	85(68%)
Klebsiella spp.	50 (1.47%)	43(86%)
Citrobacter	09 (0.03%)	6(66%)
Proteus species.	12 (0.05%)	5(41%)
Enterobacter	07 (0.02%)	5(71%)
Enterococcus species	20 (0.085%)	2(10%)
Candida species	11 (0.32%)	--
Total	234	146(62%)

Table.3 Distribution of various types of -β lactamases in Family Enterobacteriaceae

Organisms	ESBL positive	MHT positive	MBL positive	Both MHT& MBL positive	Amp C positive	Carbapenemase+ Amp C+ESBL β Lactamases.	Total
Escherichia coli(n=125)	**25(20%)**	**8(6.4%)**	**5(4%)**	**5(4%)**	**15(12%)**	**5(4%)**	**63(50.4%)**
Klebsiella pneumoniae(n=50)	**20(40%)**	**6(12%)**	**3(6%)**	**2(4%)**	**7(14%)**	**3(6%)**	**41(82%)**
Enterobacter species (n=07)	**3(42%)**	**1(14%)**	**-**	**-**		**1(14%)**	**5(71%)**
Citrobacter freundii (n=09)	**4(44%)**	**1(11%)**	**-**	**-**	**-**	**-**	**5(55.5%)**
Proteus species. (n=12)	**2(16.6%)**	**-**	**-**	**-**	**-**	**-**	**2(16.6%)**
Total -203	**54(23.7%)**	**16(6.8%)**	**8(3.4%)**	**7(2.9%)**	**22(9.4%)**	**9(2.64%)**	**112(47.8%)**

Fig.1 Novel disk placement method



1. Imipenem, 2. Cefotaxime, 3. Cefoxitin, 4. Ceftriaxone
5. Ceftazidime –clavulanic acid, 6. Ceftazidime, 7. Azetrionam

Fig.2 ESBL producer

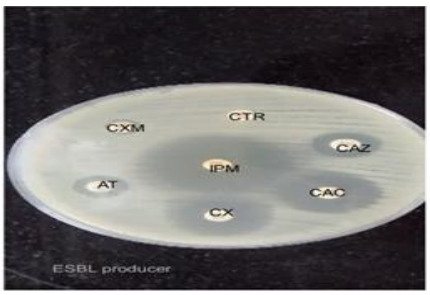


Fig.3 AMP c*b-lactamase* producer

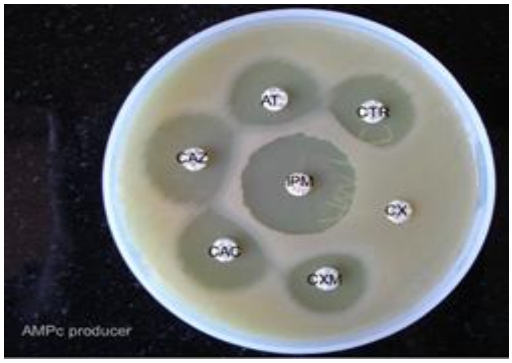


Fig.4 ESBL and AMPc producer

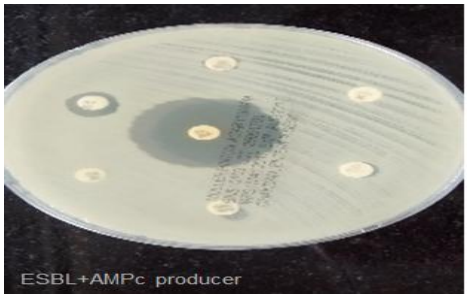


Fig.5 ESBL+ AMPc + carbapenemase producer

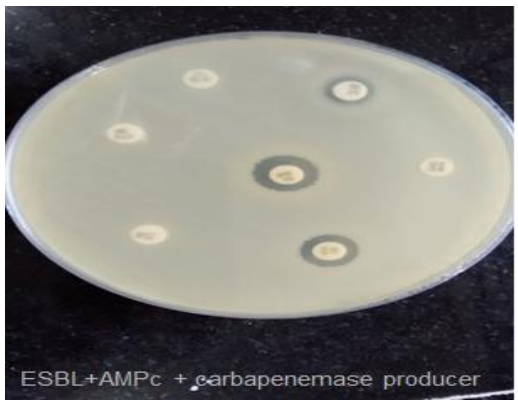
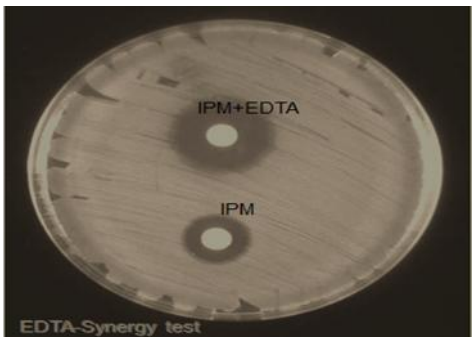


Fig.6 Modified HODGE test

Fig.7 Double disc potentiation test



Enterococcus species and Candida species *as a* rectal colonizer. 54(28.7%) isolates ESBL producing *Enterobacteriaceae,* 31(13.1%) isolates were cabapenemase producer 9(2.64%) isolates were cabapenemase +Amp C+ESBL combined producers (Table 3). Beta-lactamases also known as penicillinase are enzymes produced bacteria that provide multi resistance to β-lactam antibiotics such as penicilins cephalosporins, cephamycins, and carbapenems**.** Bacterial translocation of such multi resistant organism may be a normal phenomenon occurring on frequent basis in healthy individuals without any deleterious consequences. But when the immune system is challenged extensively, it

breaks down and results in septic complications at different sites away from the main focus. Cross transmission of the resistant strains can occur relatively easily if strong hygiene measures are not taken.

In our study 234-gram negative bacilli were isolated from 110 rectal swabs among them 146 were multi drug resistant gram-negative bacilli, among those 112(47.8%) were-β lactamases producers 54(23.7%) ESBL, 22(9.4%) Amp C, and 31(13.1%) isolates were cabapenemase producer by MHT and MBL screen and 9(2.64%) isolates were cabapenemase + Amp C + ESBL combined producers. Shamshad begum Esak *et al.,* 2015; Deshmukh *et al.,* 2011; Shanmugan *et al.,* 2013 Priya Data *et al.,* 2012; Gupta *et al.,* 2006have reported varying resistance rates of carbapenem in Enterobacteriaceae ranging from 5,75% to 51 %. Similar study by Deepa *et al.,* 2014 showed 42.8% isolates were cabapenemase producer from gut asymptomatic colonization.

In present study nasal MRSA carriage rate was8%as compared to study by Eddie Chi Man Leung *et al.,* (2013) it was 1.38%, 14% in study by A Kohlenberg *et al.,* (2011). Screening for asymptomatic MDRO colonization will reduced the prevalence of MDRO among hospitalized patients by using a variety of combined interventions like improvements in hand hygiene, use of Contact Precautions until patients are culture-negative for a target MDRO, active surveillance cultures (ASC), education, enhanced environmental cleaning, and improvements in communication about patients with MDROs within and between healthcare facilities. However, screening patients who were not receiving antimicrobials had a minimal beneficial impact. There are few limitations in the study that we screened only nasal and gut Enterobacteriaceae colonization, need to include other anatomical sites from other wards like long term care facilities and conduct larger epidemiological study.

Present study emphasis the need for strong infection control programs to detect colonization of gut and other anatomical site by multidrug resistant organisms. Need to reduce overuse of Antibiotics and establish good antibiotic stewardship programs. Implementation of screening in intensive care unit could reduce the MDRO transmission.

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