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SCREENING AND IDENTIFICATION OF MULTI-DRUG RESISTANCE NOSOCOMIAL INFECTION, ISOLATES FROM CLINICAL SPECIMEN: A CROSS-SECTIONAL STUDY

Ramsi Vakayil[1], Sivakumar Krishnamoorthy[2], Gnanendra Shanmugam[3] Senthil Kumar Nachimuthu[4] Srinivasan Ramasamy[5] and Maghimaa Mathanmohun*[6]

[1]Department of Microbiology, Muthayammal College of Arts and Science, Rasipuram, Namakkal DT. (Tamilnadu), India.
[2]Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar (Tamilnadu), India.
[3]Department of Biotechnology, Yeungnam University, Gyeongsan, Gyeongbuk, 38541, Korea.
[4]Department of Biotechnology, Mizoram University (A Central University), Aizawl (Mizoram), India.
[5]Member secretary, Tamil Nadu State Council for Science & Technology, Chennai (Tamilnadu), India.
[6*]Principal Investigator (S & T project, TNSCST), Department of Microbiology, Muthayammal College of Arts & Science, Rasipuram TK, Namakkal DT (Tamilnadu), India.

Abstract

Multi-drug resistant (MDR) nosocomial infections are a major health crisis in the world. They are the current WHO given priority for the antibiotic resistance pathogens, AMR (Antimicrobial resistance) scrutiny and finding of novel antibiotics. On the other hand, there is a lack of data on nosocomial infections caused by bacteria in and around the Namakkal District. Consequently, the present study dogged the enormity and profile of multidrug nosocomial pathogens. A hundred wound and skin specimens were collected aseptically and processed using standard bacteriological procedures by sterile cotton swabs by skilled laboratory personnel in and around Namakkal DT. The isolates were identified by microscopy and biochemical profiling. Antibacterial susceptibility assay on isolates was performed using the disk diffusion Kirby Bauer assay. The outcome of the result was interpreted as per the standard protocol. The nosocomial pathogens of wound and skin specimens were isolated from hospitals in and around Namakkal District (100 nos.) Among the 100 specimens total of 120 significant nosocomial pathogens were identified based on their colony morphology, microscopy and biochemical profiling. On the whole occurrence of the nosocomial MDR as *Staphylococcus aureus* (30 isolates), *Pseudomonas aeruginosa* (27 isolates), *Escherichia coli* (18 isolates), *Klebsiella pneumonia* (15 isolates), *K. oxytoca* (18 isolates) and *Acinetobacter baumanii* (12 isolates). According to the antibacterial activity results, all the isolates were 100% resistant against vancomycin (5µg). All six isolates were resistant to two to five antibiotics. It showed the multidrug-resistance (MDR) pattern. *K. pneumoniae* was 100% resistant against all the five antibiotics. *P. aeruginosa* and *E.coli were resistant against tetracycline, vancomycin* and cotrimoxazole. *S. aureus* was showed resistant against Chloramphenicol, *vancomycin* and cotrimoxazole. *A. baumanii* and *K. oxytoca* was resistant against two antibiotics. Multidrug resistance in nosocomial infections is the major crisis of human health in the world. Hence, imperative interference to MDR nosocomial infection deterrence practices mandatory and the patient's treatment care should be guided with antibacterial susceptibility assay.

***Key words*:** Nosocomial pathogen, Multi-drug resistant pathogens, Antibacterial activity.

Introduction

Bacterial MDR (Multidrug-resistant) infections are predictable as one of the foremost intimidation to worldwide health issues. The nosocomial infections in the world are produced by MDR bacteria (Motbainor *et al.,* 2020). Nosocomial MDR infections are the most noteworthy universal health concern. Antibiotic drugs and the agents of other antibacterial compounds are the most important to battle bacterial pathogens in creature medicine (Gyles, 2011). MDR Nosocomial pathogens intricate the medication of infections or disease and had an unpleasant result on clinical conclusion and raise the treatment costs for the patient (Solomon *et al*., 2017).

***Author for correspondence :** E-mail: mmaghimaa@gmail.com

However ridiculous use of antibacterial drugs, self-drug medication, improper over-prescription of medicine, a lengthy period of hospitalization, morbidity, mortality has led to complimentary circumstances, contact and spread of MDR strains of diverse pathogens (Morales *et al*., 2012; Manenzhe *et al.,* 2015; Huang *et al*., 2018). The infected patients are complicated to treat, because of pitiable clinical conclusion and more costs for health care (Huang *et al.,* 2018). The above mentioned adverse physical condition necessitates imperative consideration from researchers of pharmaceuticals and scientific academy to widen novel therapeutic drugs with a divergent approach for the avoidance and medication of MDR infectious diseases (Basavegowda *et al.,* 2020). However, there is a paucity of information on the lumber of nosocomial MDR infections in and around Namakkal DT. The absence of local antibiogram information correlated with patients' self-medication prescription and pitiable alertness on antibacterial resistance is a big problem of mankind. Thus, the present study was designed at determining the Multidrug resistance nosocomial pathogens.

Materials and Methods

Specimen collection

Hundred wound and skin specimens were collected aseptically by sterile cotton swabs by skilled laboratory personnel in and around Namakkal DT. The specimens were shifted to the laboratory after collection using Amies transport media (Rods, 2014).

Specimens Processing

The wound and skin swabs collected were streaked on blood agar and McConkey agar by sterile inoculation loop. The inoculated plates were incubated at 35-37°C for 24-48 hours. Preliminary detection of bacteria was based on colony characteristics of the organisms (Mama *et al*., 2014).

Physiological and biochemical analysis

The bacterial isolates were analyzed according to their microscopic and biochemical profilings similarly (Gram reaction, motility, utilization of citrate, IMVIC - indole, methyl red, Voges Proskauer, carbohydrate fermentation and urease tests) is carried out (Hung *et al.,* 2016).

Antibacterial sensitivity test

Antibacterial sensitive testing was carried out for each isolate of *E.coli, Staphylococcus aureus, Klebsiella pneumonia, K. oxtoca, A. baumannii* and *P. aeruginosa* on Mueller Hinton agar (MHA) plates (Oxoid, UK) by Kirby - Bauer disk diffusion procedure (Bauer *et al*., 1996).

All the isolates were tested against the following five antibiotics: Chloramphenicol (30µg), Tetracycline (30µg), Vancomycin (5µg), Gentamycin (10µg) and Cotrimoxazole (25µg). Through a germfree swab, the preferred colonies were spread on the muller Hinton agar plate and allowable to parched for 2-5 min. Afterward, with a disc dispenser, the antibiotic discs were kept over isolates on the MHA plates and incubated at 25-30°C for 18-24 h (Kasiwut *et al*., 2015). The zone of inhibition (ZOI) via plate ruler was measured and illustrated the isolates as Resistant (R), Intermediate (I), or Susceptible (S) (Ahtesh *et al.,* 2016; Wu *et al*., 2015). The grades were interpreted using the criterion zone sizes of the Clinical and Laboratory Standards Institute (CLSI, 2017) guidelines.

Results

Microscopic and biochemical characterization

The nosocomial pathogens from wound and skin specimens were isolated from hospitals in and around Namakkal District (100 nos.) and investigated for microscopy and biochemical characterization (Table 1 and 2). The colony morphology of these isolates was circular, small, convex, shiny, opaque, smooth colony, large, swarming growth, mucoid, dome-shaped, tiny and translucent colonies. Under microscopic assessment, gram-positive and gram-negative bacteria were too found. Biochemical studies such as IMVIC-indole, methyl red,

Table 1: Preliminary test for the isolation of nosocomial pathogens.

Gram Staining	Motility	Isolates
G +ve Cocci in clusters	Non-motile	1
G –ve Rod	Non-motile	2
G –ve Rod	motile	3
G –ve Rod	Motile	4
G –ve Rod	Non-Motile	5
G –ve Rod	Non-Motile	6

Table 2: Biochemical characterization of the nosocomial pathogenic isolates.

Isolates	Carbohydrate Fermentation				I	MR	VP	CIT	Urease	Proposed Organisms
	G	L	S	M						
1	P	N	P	P	N	P	N	N	N	*Staphylococcus aureus*
2	P	P	P	P	N	N	P	P	P	*Klebsiella sp.*
3	P	N	N	P	N	N	N	P	P	*Pseudomonas aeroginosa*
4	P	P	P	P	N	P	N	N	P	*E. coli*
5	P	N	N	N	N	N	N	P	N	*Acinetobacter baumanii*
6	P	P	P	P	P	N	P	P	P	*Klebsiella oxytoca*

I: Indoel; MR: Methyl Red; VP: Voges Proskauer; CIT: Citrate; G: Glucose; L: Lactose; S: Sucrose; M: Mannose; P: Postive; N: Negative.

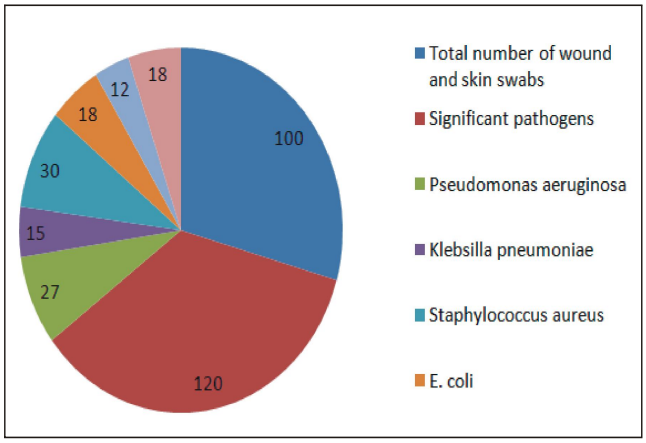

Fig. 1: Screening of nosocomial from clinical samples.

Table 3: Screening of nosocomial pathogens from clinical samples.

Total number of wound and skin swabs	100
Significant pathogens	120
Pseudomonas aeruginosa	27
Klebsiella pneumoniae	15
Staphylococcus aureus	30
E. coli	18
Acinetobacter baumanii	12
Klebsiella oxytoca	18

Voges Proskauer, carbohydrate test, citrate utilization and urease test results were given (Table 2). Among the 100 samples total of 120 significant nosocomial pathogens were identified (Table 2 and Fig. 1). The overall prevalence of the pooled nosocomial MDR as *Staphylococcus aureus* (30 isolates), *Pseudomonas aeruginosa* (27 isolates), *E. coli* (18 isolates), *Klebsiella pneumonia* (15 isolates), *K. oxytoca* (18 isolates) and *Acinetobacter baumanii* (12 isolates).

Antibacterial activity

According to the antibacterial activity results, the sensitive zone of inhibition for Chloramphenicol (30µg) was sensitive to *K. oxytoca* (23mm), *P. aeruginosa* (18mm) and *E.coli* (18mm), Tetracycline (30µg) was observed on *Staphylococcus aureus* (26mm), *A. baumanii* (21mm) and *K. oxytoca* (21mm) only, Gentamycin was sensitive to *A. baumanii* (20mm), *Staphylococcus aureus* (19mm), *P. aeruginosa* (18mm) and *E.coli* (18mm). Cotrimaxazale (25 µg) was sensitive to *A. baumanii* (22mm) and *K. oxytoca* (21mm). The zone of inhibition showed by the antibiotic against the 6 isolates and the results are measured and tabulated (Table 4 and Fig. 2). All the pathogens showed a resistant pattern on vancomycin (5µg). Most of the organisms showed a multidrug-resistant pattern.

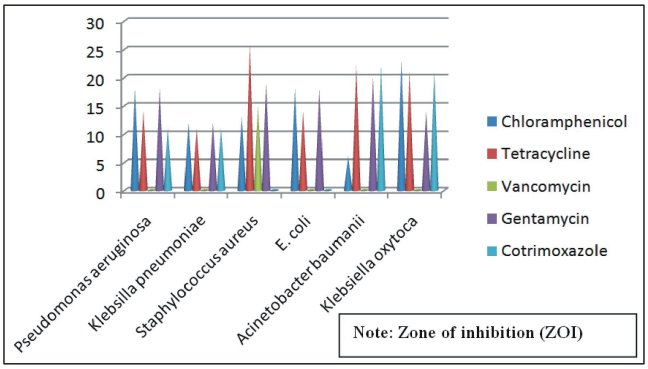

Fig. 2: Multi-drug resistant pattern of Antibiotic-sensitivity test against the nosocomial pathogens.

Multi-drug resistance profiles of the nosocomial pathogens

Staphylococcus aureus, P. aeruginosa, *E. coli*, *K. pneumonia, K. oxytoca* and *A. baumannii* isolates were resistant to two to five antibiotics. It showed the multidrug-resistance (MDR).

Discussion

Drug-resistant nosocomial infections are a flattering severe problem in health care and hospital care, rising morbidity, mortality, extent the stay and increase the costs of health care (Exner *et al*., 2017; Johnson, 2015). The drug resistance profiles of nosocomial infections showed fluctuations amid hospitals in the earth. Most of the bacterial infections are resistant to multiple antibiotic drugs (Johnson, 2015; Asres *et al*., 2017). This study showed the nosocomial infections owing to six MDR

Table 4: Antibiotic sensitivity test against the nosocomial pathogens (ZOI in mm).

Nosocomial pathogens	C - 30µg		Te 30µg		Va-5µg		Gm-10µg		Ctx -25µg	
	ZOI	Inf	ZOI	Inf	ZOI	Inf	ZOI	Inf	ZOI	Inf
Pseudomonas aeruginosa	18±1	S	14±1	R	-	R	18±1	S	11±1	R
Klebsiella pneumoniae	12±1	R	11±1	R	-	R	12±1	R	11±1	R
Staphylococcus aureus	13±1	R	26±1	S	15±1	R	19±1	S	-	R
E. coli	18±1	S	14±1	R	-	R	18±1	S	-	R
Acinetobacter baumanii	6±1	R	22±1	S	-	R	20±1	S	22±1	S
Klebsiella oxytoca	23±1	S	21±1	S	-	R	14±1	R	21±1	S

R: Resistance; I: Intermediate; S: Sensitivity; ZOI: Zone of Inhibition; Inf: Inference;
C: Chloramphenicol; Te: Tetracycline; Va: Vancomycin; Gm: Gentamycin; Ctx: Cotrimoxazole.

bacteria in wound and skin specimens. In the present study, Among the 100 samples total of 120 significant nosocomial pathogens were identified. The overall prevalence of the combined nosocomial MDR as *Staphylococcus aureus* (30 isolates), *Pseudomonas aeruginosa* (27 isolates), *E. coli* (18 isolates), *Klebsiella pneumonia* (15 isolates), *K. oxytoca* (18 isolates) and *Acinetobacter baumanii* (12 isolates). This indicated that MDR nosocomial infections are the main health crisis. Elevated load of patient, excess numbers, poor infection control practices, self-drug medication, consumption and ludicrous use of antibiotic drugs, inappropriate over-prescription of drugs, a prolonged time of hospitalization divergence in aseptic measures might be the reason. This result was consistent with the findings of (Asres *et al*., 2017; Kateete *et al*., 2017; Tolera *et al*., 2018). In contrast, the overall nosocomial MDR pathogen in the present study varied than (Xu *et al.,* 2015; Tolera *et al*., 2018). This may be owing to deviation in the size of the specimen, patient age, a moment of stay in the hospital, site of infection, microbiological assays engaged for identification of MDR nosocomial strains. Furthermore, the lofty self-prescription practice of antibiotic drugs and the use of antibiotic drugs exterior the hospital may add the resistance rate higher to diverse classes of antibiotic drugs.

Conclusion

Frightening fraction increase of multidrug resistance in nosocomial infection reported in the present study. All six isolates were resistant to at least two antibiotics. Hence, imperative interference to MDR nosocomial infection deterrence practices mandatory and the patient's treatment care should be guided with antibacterial susceptibility assay.

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