



Molecular Characterization of Isolated Multidrug Resistant Bacteria from Tertiary Care Hospitals of Ahmedabad: Comparison between Pre-Covid-19 and Current Scenario

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Abstract

Background: Hospital Acquired Infections (HAIs) are a major concern for healthcare setups, as it increases the overall cost of treatment, patients stay in hospitals making them susceptible to secondary and tertiary infections, and in few unfortunate cases HAIs result in mortality.

Purpose: To prevent or control HAIs, the process of evaluating the organisms isolated from the critically maintained area is considered of epitome importance and everlasting practice in the healthcare industry. Identifying such organisms and screening them for antibiotic resistance is not only mandatory; but it also helps professionals to understand trends of colonization, if any.

Methods: Sensitive areas of healthcare setups were screened on a monthly basis from years 2017 to 2020. Total of 4400 samples of hospital hygiene were collected. Multidrug-resistant organisms were subjected to molecular characterization to detect the presence of carbapenem genes.

Results: Evaluation data of both, pre and during Corona Virus Disease COVID-19 era were compared. And it was found that the prevalence of pathogenic and/or non-pathogenic strains in healthcare setups decreased drastically. It is possible only because of the awareness generated in laymen as well as healthcare workers due to unforeseen critical situation ultimately proving to be a blessing for the future generation.

Keywords: Hospital Acquired Infections (HAIs); OT; ICU; Pathogen; Microbiology surveillance; Carbapenem resistance

Introduction

Hospital-acquired infections are a menace to the healthcare industry and have proven to be life-threatening [1]. Regular microbiological surveillance of sensitive areas provides important information related to commonly isolated organisms; the data is utilized to decide healthcare policies [1]. Clues help specialists to predict infection trends, development of resistance, or colonization [2]. COVID-19 has changed many things we perceived differently especially in the field of healthcare hygiene [3]. Because of uncertainty, healthcare workers were sceptical and were forced to follow the regime strictly. Healthcare workers and patients were forced to maintain distance; patients' access was highly restricted, hands were sanitized or disinfected most frequently, faces were covered with masks, PPE kits were worn; utmost care was taken by the Corona Front Line Warriors. Amid this chaos, people developed insight about critical aspects of healthcare viz. infection, epidemic, pandemic, contamination, hygiene, sanitization and disinfection, surface disinfection etcetera; however, healthcare, per se, has benefitted the most from this situation in the terms of awareness [4]. All this was possible because of the awareness generated from current pandemic and infection control specialists found every one following the basics. Healthcare workers have followed every single guideline and have fought with current national, or rather international crisis, with the best of their ability. Due to this, the rate of isolating multidrug resistant organisms from healthcare setup has drastically decreased [5].

Materials and Methods

The study does not involve samples of human origin; hence, requirement follow the norms of the declaration of Helsinki is not required. No medicines or placebo were prescribed. The resistance screened was performed against established formulas only. In this study, surveillance tests from the critically important wards of tertiary care hospitals of Ahmedabad were taken and analysed between the years 2017 till July 2020. Samples from critically important ward were collected bi-monthly and those with less importance were collected on monthly basis. Accordingly, to the CLSI guideline, asepsis was maintained while collecting air samples and surface swabs were collected from frequently used surfaces by making virtual marking of 5 × 5 square centimetre area [6]. Swabs were enriched with nutrient broth and were inoculated on Blood agar and MacConkey agar and incubated at 37°C for 24 hours in the laboratory [6]. Air sampling was carried out using air sampler (active sampling) from Hi-media using air sampler LA002, having highest sampling volume of 2520 litres of air max and requiring 3.5 minutes to sample 1000 litres of air having a flow rate of 280 litres/min with particle cut off size of >3 μm [7]. Blood agar (BA), MacConkey agar (Mac), Standard plate count agar (SPC) and Sabouraud Dextrose Agar (SDA) were inoculated. Air passed from the air sampler is blown on the strips [7]. Colonies, growing as a result of the presence of microorganisms, are counted and processed further for identification and susceptibility testing. Molecular characterization was carried

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out of carbapenem producing organisms. Antibiotic resistance screening was carried out using method provided by CLSI and confirmatory tests, like Modified hodge test and combined disc tests, were carried out before proceeding ahead with genetic characterization [8]. Molecular characterization was carried out, after extracting bacterial whole ge-

nomics DNA, using ready to use primers obtained from ThermoFisher Scientific on 7500 real-time PCR instrument for carbapenem genes, viz. blaNDM-1, blaIMP-11, blaVIM-2 and blaKPC-3 having catalogue numbers Taqman Pa04646121_S1, Pa04646119_S1, Pa04646155_S1 and Pa04646152_S1 [9].

Sr. No.	Sampling time	Sample	Organism Wise Distribution of sample collected before and after March 2020								Total
			AMC	Y & M	E. coli	<i>Pseudomonas spp.</i>	<i>S. aureus</i> Co. +ve	<i>S. aureus</i> Co. -ve	<i>Bacillus spp.</i>	<i>Klebsiella spp.</i>	
1	Pre CO-VID	Air	880	40	85	60	50	250	660	5	2030
2	Post COVID	Air	10	6	0	0	0	5	10	1	32
3	Pre CO-VID	Surface Swab	1211	450	135	40	10	410	820	24	3100
4	Post COVID	Surface Swab	50	30	5	2	1	10	13	6	117

Table 1: Pre and Post COVID air and swab samples organism wise analysis

Results

From Table 1, one could fathom that out of total of 4400 samples 2403 surface swabs and 804 air samples were collected before March 2020, and 1055 surface swabs and 138 air samples were collected after March 2020. Total of 5279 organisms were isolated from total number of samples screened during this period, pathogenic or non-pathogenic; however, the ratio of isolation of organisms before and after March 2020 drastically decreased due to the facts mentioned earlier.

Out of total strains screened for antibiotic resistance, 4795 isolates were found non-pathogenic; whereas, 484 organisms were found to be resistant to more than one group of antibiotics, multidrug resistant strains. With the help of confirmatory tests, twenty five strains of *Klebsiella spp.*, *Pseudomonas spp.* and *E. coli* all together were selected for molecular characterization to detect presence of carbapenem producing genes (Figure 1).

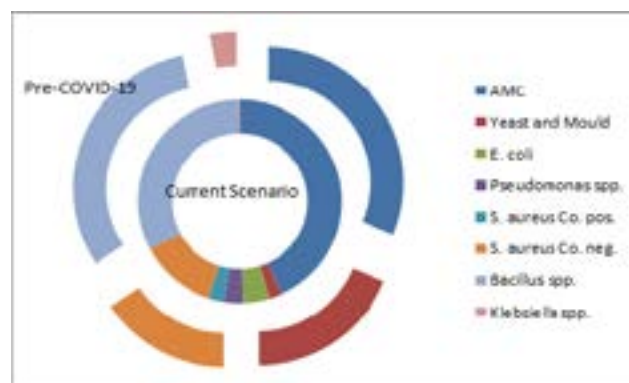


Figure 1: The graphical representation in the chart shows pre-COVID and current situation comparison of organisms isolated through air sampling

Tested Organism	Total number of isolates		blaNDM-1	blaIMP-11	blaVIM-2	blaKPC-3
	Pre-COVID-19	Current Scenario				
<i>E. coli</i>	9	1	2	1	1	3
<i>Klebsiella spp.</i>	8	2	5	4	6	4
<i>Pseudomonas spp.</i>	4	1	2	1	1	3

Table 2: Molecular characterization of carbapenem producers to detect the carbapenem gene presence

From the Table 2, it is evident that the presence of multiple genes was observed in the isolates of *Klebsiella spp.* and *Pseudomonas spp.* Most frequently found carbapenem genes were blaNDM-1 and blaKPC-3.

The strains with the presence of multiple genes are being analysed further for gene expression analysis and by sequencing the whole genome (Figure 2).

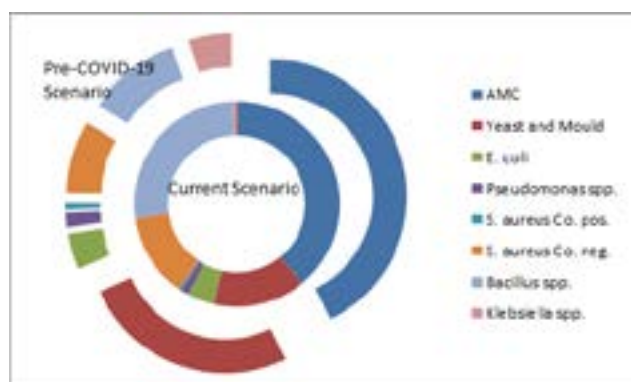


Figure 2: The graphical representation in the chart represents isolation data of pre-COVID and current situation comparison of organisms isolated through surface swab sampling

Discussion

In-depth analysis of sensitive areas of healthcare setup is inevitable; guidelines must be followed in maintaining the same, for it plays an important role in restricting hospital acquired infections (HAIs) [10-12]. As per the WHO Guidelines to Laboratory Method, OT is considered well maintained when bacterial load is found to be lower than 180/m³ of air [2,13]. From the present study it is evident that though the count was below in pre COVID-19 era, it was at far more low level after following stringent guidelines.

The purpose of the study was to highlight that in the current critical situation when guidelines are stringently followed, the overall presence of organisms was minimal, and reason behind it could be frequent cleaning of various surfaces and hands using sanitizers and disinfectants or minimal access to the patients.

Molecular characterization confirmed the presence of organisms bearing multiple genes isolated from sampling carried out prior to the COVID-19 pandemic. Though further analysis is pending of these isolates, it is evident from the post-COVID-19 data that the overall count of such organisms has decreased drastically, proving to be beneficial to patients and healthcare setups both.

Conclusion

It is in human nature to adjust to any situation, so have we during this unprecedented event in the history of mankind; however, with the attained knowledge from every such experience people evolve. An effort is made by sharing this research to understand the fact that implantation and maintenance of stringent quality control procedure is possible by every healthcare professional and could provide extraordinary out-

comes.

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