ORIGINAL ARTICLE

CHANGING TRENDS OF MULTIDRUG RESISTANT BACTERIA: A SIX YEAR EXPERIENCE FROM A TERTIARY CARE HOSPITAL

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Background: There is a continuous increase in the number of bacteria showing resistance to various antibiotics, limiting the treatment options for infections. The objective of this study was to assess the trend in resistance pattern of multi drug resistant organisms over a period of 6 years. **Methods:** A retrospective study was conducted in Indus Hospital and Health Network, Karachi, Pakistan from January 2014 to December 2019. Multidrug resistant organisms were isolated from various samples and the data of corresponding patients were extracted from electronic medical record. The patients of all age groups and either gender was included. Specimens were inoculated on Sheep Blood Agar, chocolate agar and MacConkey agar. Organisms were identified and antibiotic susceptibility testing was performed according to Clinical Laboratory Standard Institute guidelines. **Results:** In 34628 cases, 5159 (14.8%) were isolated as MDR organisms. Out of these 44.2% were Gram negative, while 55.7% were Gram-positive bacteria. The highest MDR trend was observed for *A. baumannii* (0–70%) followed by MRSA (0–64%) *P. aeruginosa* (0–16%) Enterococcus (0–10%) CRE (2.8–5.8%). **Conclusion:** The continuous rising trend of multidrug resistant organisms has been observed during the period of our study. Therefore, there is an imperative need of constant monitoring and firm adherences to infection control strategies to avoid spread of MDR organisms.

Keywords: Multidrug resistance; Gram negative bacteria; Gram positive bacteria

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INTRODUCTION

Multidrug resistant organisms (MDROs) are defined as microorganisms, predominantly bacteria, that are resistant to one or more classes of antimicrobial agents.[1] Antimicrobial resistance is a self-defence mechanism of bacteria in order to combat antibacterial drugs. They alter proteins, and modify their membrane permeability to escape from lethal antibiotic effects.[2] Over the counter availability and continuous use of antibiotics accounts for ineffectiveness of antibiotics, leading to a global rise in drug-resistant bacteria.[3] Globally MDR bacteria is responsible for 700,000 deaths every year and it is expected that this figure will rise to 10 million by 2050, along with huge economic loss.[4] The Infectious Diseases Society of America (IDSA) declares MDROs as one of the greatest threat, to human health worldwide.[5]

It has spread to too many countries and regions, including Pakistan, owing to the indiscriminate use of antibiotics and poor infection control measures. The growing burden of antimicrobial resistance (AMR) in Pakistan have been highlighted over the last few years. Although there has been a growing literature on AMR but currently no national systematic data is available on AMR.

In recent years, several microbial infections are no longer treatable with commonly used antimicrobial drugs due to the increase in MDR. Common bacteria that show MDR pattern are methicillin-resistant *Staphylococcus aureus* (MRSA), *vancomycin resistant Enterococcus* (VRE), *carbapenem-resistant Enterobacteriaceae* (CRE), *carbapenem-resistant Acinetobacter baumannii* (*A. baumannii*) and *Pseudomonas aeruginosa (P. aeruginosa)*.[6] Typically, MDR bacteria are associated with nosocomial infections. However, some are responsible for community-acquired infections (CAI) as well. This subsequently boosts the number of infections and put the large population at risk. In addition, when the incidence of resistance pattern in bacteria causing CAI exceeds a specific threshold, broad spectrum antibacterial or combination of antibiotics are prescribed for the empiric treatment of CAI.[7]

To overcome this emerging problem and to identify outbreaks it is necessary to develop, improve, coordinate and monitor surveillance capacity. It can also be helpful in developing stewardship initiatives. All these activities would

contribute to optimize and reduce AMR burden and ultimately improve population health while preserving the efficacy of existing antimicrobial drugs.[8] In this study, we reviewed the trends and epidemiology of various MDR bacteria in a tertiary care hospital of Karachi, Pakistan.

MATERIAL AND METHODS

This retrospective study was conducted in the section of Microbiology at the Indus Hospital and Health Network Karachi from January 2014 to December 2019; to evaluate the frequency of MDROs in our community. Bacterial isolates were recovered from all samples received in the laboratory during the study period including blood, urine, wounds and respiratory samples (sputum, tracheal aspirates, etc). Samples from both genders and patients of all ages were included in the study. The specimens were collected and processed according to standard operating procedure.[9] Repeat isolate during a month (30 days) from same patient and sample received in an unsterile container were excluded from the study. Antibiotic susceptibility testing was performed according to Clinical laboratory standard institute 19 (CLSI).[10] Exclusion criteria was fulfilled. Written approval was taken from the institutional ethical committee.

Specimens were inoculated on Sheep Blood Agar (SBA), Chocolate and MacConkey agar and smear were prepared for gram staining. Organisms grown on culture were identified based on biochemical reactions and analytical profile index system (API). Antibiotic susceptibility testing was performed by Kirby Bauer disc diffusion method using Mueller Hinton agar plates and SB–Mueller-Hinton agar (SBMHA) as suggested by the CLSI and British society for antimicrobial chemotherapy BSAC.[11] The antimicrobial discs were obtained from Oxoid®, UK and Bioanalyse®, Turkey. MDR bacteria were considered to be resistant to at least one antibiotic in case of MRSA and VRE and two groups of antimicrobials for Gram negative bacteria was considered for inclusion of the organism as MDR.[12] The following quality control (QC) strains were included: *S. aureus*ATCC25923, *E. coli* ATCC25922, *K. pneumonia* ATCC700603, and *P.aeruginosa* ATCC27853.

Data was analyzed using SPSS version 24.0. Qualitative variables such as microorganisms, sex and antibiotic susceptibility, frequencies and percentages were calculated. For quantitative variables such as age and site of specimen, mean and standard deviation was considered.

RESULTS

The distribution of various sites of specimen is mentioned in Table1, most of the samples in this cohort are from respiratory tract. The total number of organisms isolated from different samples over the period of six years were 34628. Among these organisms *Enterobacteriaceae* was the most common. Of these 34628 organisms 5159 (14.8%) were found to be MDR with 44.2% Gram-negative and 55.7% Gram-positive bacteria (Figure-1). The frequency of overall MDR cases was 1.9% in 2014, 1.3% in 2015, 18.6 in 2016, 17.9.0% in 2017, 18.9% in 2018 and 23.6% in 2019 (Figure-3).

The frequency of gram-negative bacteria was found to be (11.3%) in 2014, (14.3%) in 2015, (16.8%) in 2016, (21.1%) in 2017, (25.4%) in 2018 and (10.9%) in 2019 (Figure-2). Different Gram-negative organisms and their percentages were as follow, *Enterobacteriaceae (*62.2%)> *P. aeruginosa (*9.4%), >*A. baumannii* (4.1%).

The frequency of MDR gram-negative bacterial cases increased over the period of six years (Figure-2). from 2.3% (2014) to 12.1% (2019) with 1.6% (2015), 10.3% (2016), 11.2% (2017) and 10.8% (2018). In particular, increasing trend has been recorded for MDR *A.baumannii* which has risen from 0–70%, followed by *P.auroginosa* (0–16%) and CRE (2.8–5.8%) during the study period (Figure-3).

S. aureus and *Enterococcus* were two gram-positive organisms isolated from clinical samples with *S. aureus* as predominant one (17.5%) followed by *Enterococcus* (6.5%). The overall frequency of Gram-positive bacteria was found to be (9.2%) in 2014, (12.3%) in 2015, (12.1%) in 2016, (23%) in 2017, (29.6%) in 2018 and (13.6%) in 2019 (Figure-2). The frequency of MDR gram-positive bacteria increased in the following manner with (0%) in 2014, (0.09%) in 2015, (54%) in 2016, (37.3%) in 2017, (40.4%) in 2018 and (52%) in 2019 (Figure-2). In precise, increasing trend has been observed for MRSA, which has risen from 0% (2014) to 64% (2019) followed by VRE (0 to 10.8%) (Figure-3)

Table-1: Distribution of specimen

Sites of Specimen	Numbers	Percentages
Wound	247	23
Blood	321	30
Respiratory tract sample	412	38
Urine	97	9

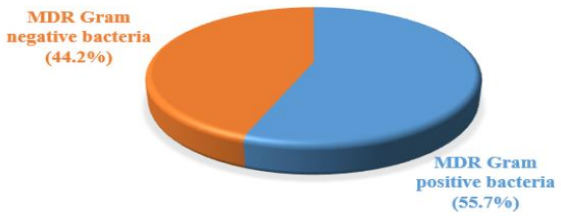


Figure-1: Frequency of Isolation of MDR organisms

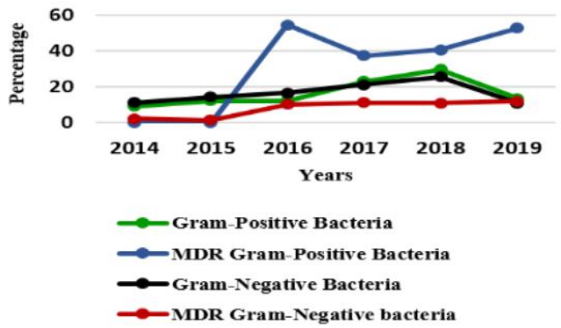
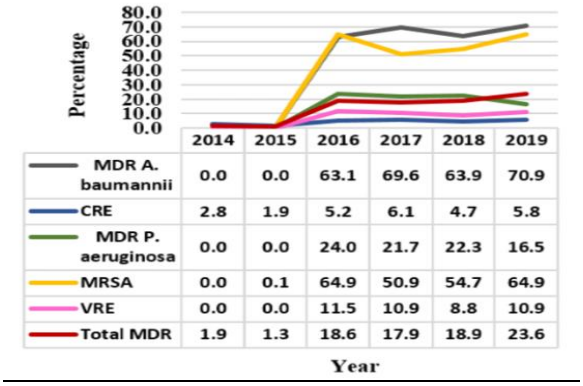


Figure-2: Annual Trend of Total and MDR positive organism



	2014	2015	2016	2017	2018	2019
MDR A. baumannii	0.0	0.0	63.1	69.6	63.9	70.9
CRE	2.8	1.9	5.2	6.1	4.7	5.8
MDR P. aeruginosa	0.0	0.0	24.0	21.7	22.3	16.5
MRSA	0.0	0.1	64.9	50.9	54.7	64.9
VRE	0.0	0.0	11.5	10.9	8.8	10.9
Total MDR	1.9	1.3	18.6	17.9	18.9	23.6

Figure-3: Annual Trend of MDR Organisms

DISCUSSION

Under the non-favourable conditions' microbes acquire certain characteristics that are the determinant of their survival and growth. Antibiotic resistance is of prime importance in this regard. The worldwide increase in the MDROs is a major challenge for pharmaceutical and biomedical industries. MDR bacterial infections are responsible for growing rate of mortality, morbidity and cost of prolonged treatments.[3] Over the years, antimicrobial resistance (AMR) has manifested itself as a major public health threat in many countries, including Pakistan. Recently CDC has specified that the world is on the edge of entering the ''post-antibiotic era'', where bacterial infections cause more deaths than cancer.[14] Continued Epidemiologic analysis of antimicrobial resistance is requisite for empirically treating infections, more pertinent antimicrobial prescription, new treatment alternatives and inhibiting the spread of MDR microorganisms.

In this study, the trend of incidences of MDR organism in various clinical samples, such as urine, blood, wound and respiratory infections is monitored. We have observed that the burden of overall MDROs continuously rising from 2014–2019. Our results are consistent with the data reported by various surveillance studies conducted in Africa, America, Europe, Asia, and Western Pacific region. According to these studies there is an alarming high number of MDROs have been evolved with respect to time.[15] Interestingly we found that MDR gram positive bacteria are considerably higher as compared to MDR gram-negative bacteria. This is in contrast with the findings reported in Italy where MDR gram negative bacteria were predominant than gram-positive.[16]

The burden increased between 2014–2019 for four of the pathogens examined in this analysis, but the incidence did not increase for all pathogens. Trend of MDR *P. aeruginosa* increased from 2014 -2018 and dropped in 2019.

Possible explanation of rising trend of MDROs is that our hospital is a charitable tertiary care centre and patients from nearby districts and even villages are admitted for treatment. Before attending the hospital, most of the patients receive different antibiotics prescribed by local general physicians often in improper dose. We also cannot ignore the lack of infection control measures required to decrease the transmission of infection in health care settings. However, the reason behind the decline in the trend observed for MDR *P. aeruginosa* remains ambiguous.

Among the five studied organisms *A. baumannii* was at the top of the list with 70% increase. The global data also indicated that community and hospital-acquired infection (HAIs) and the prevalence of institutional outbreaks due to *A. baumannii* has significantly increased in the past years particularly in ICUs.[17] Antimicrobial susceptibility pattern of *A.baumannii* is different in different regions however resistance was high in many regions.[18] The prevalence of MDR *A. baumannii* in a study carried out in Nepal was extensively high 91.0%. In another study conducted by Shrestha and Mishra approximately 96% and 95% of isolates were MDR, respectively.[19]

We also observed an escalation in the incidence of CRE. The results are consistent with the findings reported in Lebanon (20) where they also reported low increase in occurrence rate of CRE in comparison with other MDR bugs during eight years of their study. Other cities of the country, such as one of the studies conducted in Rawalpindi reported increasing occurrence of CRE Gram-negative bacteria (*E. coli* and *K. pneumonia)* with a prevalence of 17%.[21]

The increased in emergence of CRE strainsfrom 1.6% (2001) to 10.4% (2011) has also been reported in United States (US). Moreover, increase resistance to carbapenem in Enterobacteriaceae globally creates an important public health threat.[22] According to the recent data from the antimicrobial surveillance network of China, resistance rates of *K. pneumoniae* were eight times increase as compared to 2005. CR*K. pneumoniae* has also been listed in the priority microorganism by the World Health Organization.[23] According to the study done in US 34000 CRE infections occur every year in the US and 3 million infection worldwide in 2014.[24] Our results are in accordance with several studies, which also reported increase trend of CRE.[25]

As far as MDR-Gram positive bacteria is concerned the most prevalent one in our study was MRSA. It was observed to be strikingly increase during our study period (0–64%). Emergence of MRSA is a global problem in both clinical and community sites, with varying frequency among different countries. In Pakistan, 42–51% prevalence of MRSA has been observed.[26] A study designed by Naeem *et al.* in Peshawar stated 31.5% frequency of MRSA.[27] Our data showed that the number of MRSA is continuously increasing which is in accordance with the previous data, reported high rate of MRSA incidences.[28] On the contrary, studies conducted in developed countries reduction in the incidence of MRSA has been observed.[29] It could be credited to effective surveillance and treatment strategies for MRSA infections.

Other MDR Gram positive bacteria isolated from our samples was VRE but the occurrence was not found to be as high (0–10.89%) as it is observed for MRSA. The global increase in prevalence of VRE has been reported since its emergence in the 1980s. A similar increase in the prevalence of VRE, has been observed previously in different studies reported from various countries.[30]

There are certain limitations as this work is retrospective in nature it could not be determined whether the infections were community acquired or nosocomial. Hence MDR frequency was generalized for whole study population. Moreover, in this study, samples were collected from only one hospital, therefore it might not effectively signify resistance rate in public health setups.

CONCLUSION

Present study demonstrated an increasing trend of MDROs in our healthcare setup (2014–2019) specifically *A. baumannii* from the Gram-negative isolates followed by *P. aeruginosa*. Furthermore, MDR Gram positive bacteria also revealed an increasing trend of antibiotic resistance with MRSA being the prominent bug followed by VRE.

RECOMMENDATIONS

Because of the devasting impact of this problem on human lives and public health future strategies should be developed based on multifaceted collaboration among all relevant stakeholder in the region. Therefore, concerted strategies such as active surveillance, infection control programs, consistent antibiotic policies in hospitals and various sectors should be executed, which will be effective to tackle the spread of MDR strains. MDR gram negative bacteria revealed increasing trend of resistance to antibiotics, *A. aaumanni* was the leading bug in this group, followed by *P.aerugenosa*. MDR gram positive bacteria also revealed an increasing trend of antibiotic resistance and MRSA was the leading bug in this group followed by VRE

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Conflicts of interest: The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethics approval: The study was approved by The Indus Hospital ethics committee IRB (Institutional Review Board) and certified that the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Consent: This is a retrospective study; Electronic medical records have been reviewed for a six years' period. It was not possible to take consent from the patients. However, all possible efforts are made to ensure data confidentiality.

AUTHORS' CONTRIBUTION

FA: Made substantial contributions to the design of the work, drafted the manuscript and revised it critically. NK: Design the study, reviewed it critically. MK: The acquisition of data. MMA: Interpretation of data, drafted manuscript. SH: Extraction of data. NM: Revised it critically for important intellectual content.

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