BURDEN OF MULTI - DRUG RESISTANT, EXTENSIVELY - DRUG RESISTANT AND PAN - DRUG RESISTANT SUPERBUGS ISOLATED FROM VARIOUS INDOOR MICROBIOLOGICAL SPECIMENS AT TERTIARY CARE CENTERS RAWALPINDI

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ABSTRACT

Objective: To estimate burden of multi drug resistant, extensively drug resistant and pan drug resistant superbugs isolated from various indoor microbiological specimen at tertiary care centers Rawalpindi.
Study Design: Cross sectional study.
Place and Duration of Study: Department of Microbiology in Institute of Pathology, from Jul 2017 to Jun 2018.
Methodology: A total of 6126 bacteria isolated from various indoor microbiological specimen were included in the study. All specimen were collected aseptically and bacterial cultures were processed as per standard microbiological practices. Bacterial isolates were identified by using VITEK 2 systems-Version 08.01 (bio Merieux, France).
Results: Out of 6126 bacteria isolated 76% were multi drug resistant, 44% were extensively drug resistant and 1.2% were pan drug resistant. About 83% and 3.8% and 0% of bacteria isolated from pus/tissue, 67%, 58%, 0.8% of bacteria recovered from urine, 72%, 52% and 4.5% of bacteria isolated from respiratory samples and 83%, 42% and 3.4%bacteria isolated from blood were multidrug resistant, extensively drug resistant and pan drug resistant respectively.
Conclusion: The burden of MDR, extensively drug resistant and pan drug resistant among bacteria isolated from various indoor microbiological specimen is disturbingly high and highlights a serious public health problem. Coordinated efforts to implement new policies, renew research efforts and investment into new antimicrobials are needed to manage drug resistant crises.

Keywords: Antibiotic susceptibility testing, Extensively drug resistant, Multidrug resistant, Microbiological specimen, Pan drug resistant, Superbugs.

INTRODUCTION

The escalation of drug resistance to multiple antimicrobial agents in pathogenic bacteria poses a global threat to public health, as it limits the armamentarium of therapeutic agents against these life threatening bacterial infections[1]. In 2011, WHO declared "combat drug resistance: no action today, no cure tomorrow[2]". The rapid emergence of resistant bacteria has been described by many public health agencies as a "crises" or "nightmare scenario" that could have "devastating effects"[3]. The infections caused by these superbugs are associated with greater morbidity, mortality and inflicts huge health care cost[4]. Studies have shown that antimicrobial resistance is more pronounced in developing countries due to limited antibiotic options, poor quality of drugs, improper sanitation, malnutrition and debilitated healthcare systems[5]. The irrational use of antibiotics is another important factor that contributes to the antimicrobial resistance. In addition to this inherent inclination of physicians to prescribe potent antibiotics i.e. use of canon when the gun can be used to kill the same enemy is also responsible for alarming drug resistance[6].

Multi drug resistant bacteria (MDR) are now wide spread all over the world. A considerable number of extensively drug resistant (XDR) and pan drug resistant (PDR) bacteria are also being increasingly reported[7]. Amongst Gram-positive

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bacteria, emergence of resistant *S. aureus* and *Enterococcus spp* poses the therapeutic cha-llenge[8]. Gram-negative pathogens are even more problematic because of the pandemic of MDR and alarming rates of PDR are leading us towards pre-antibiotic era[9]. Although most studies come from industrialized countries, but the rate of infection may even be higher in developing countries from where there is a scarcity of data. The objective of our study was to estimate burden of MDR, XDR and PDR isolated from various indoor microbiological specimen at tertiary care centers of Rawalpindi.

METHODOLOGY

This was a cross sectional study conducted at the department of Microbiology in Institute of Pathology from July 2017 to June 2018. All bacterial culture positive microbiological specimens from indoor patients irrespective of age and gender were included in the study. Poor quality and contaminated samples were excluded from the study. Permission was sought from Institutional Ethical Committee.

Sample size was calculated using WHO sample size calculator, using specified absolute precision of 0.01, 95% confidence level and 0.138[10] anticipated population proportion. Estimated sample size was 4570 which was exceeded during our study period and a total of 6126 bacteria isolated from various indoor microbiological samples were included in the study. Microbiological specimens including wound cultures like pus and tissues, urine, lower respiratory specimens (like sputum, tracheal isolates, endobronchial washings and bronchoscopic alveolar lavage) and blood cultures received from patients admitted at tertiary care hospitals of Rawalpindi were included in the study. All specimens were collected by aseptic technique and bacterial cultures were processed as per standard microbiological practices.

Identification of Isolates: Bacterial isolates were identified on the basis of colony morphology, Gram staining, basic biochemical tests, API 20E, API 20NE and VITEK 2 systems Version 08.01 (bio Merieux, France).

Antibiotic Susceptibility Testing: The antimicrobial susceptibility profile of different bacteria was performed by modified kirby bauer disc diffusion method and by VITEK 2 systems ver. 8.01 (bio Merieux, France) using clinical laboratory standards institute (CLSI) guidelines[11].

According to standardized International definitions created by European Centre for Disease Control (ECDC) and Centre for Disease Control and Prevention (CDC), MDR was defined as resistant to at least one agent in three or more antimicrobial categories. XDR was defined as resistant to at least one agent in all but two or fewer antimicrobial categories remain susceptible. PDR was defined as resistant to all agents in all antimicrobial categories[12]. In order to ensure correct application of these definitions, bacterial isolates were tested against all or nearly all of the antimicrobial agents within antimicrobial categories as recommended by CLSI[11]. MDR bacteria can be XDR and PDR, similarly XDR bacteria can be PDR but not vice versa. Data was analyzed using SPSS version 23. Descriptive statistics were used to describe the result.

RESULTS

Out of 6126 bacteria isolated from various indoor microbiological samples 4679 (76%) were MDR, 2721 (44%) were XDR and 72 (1.2%) were PDR. Out of isolated bacteria 1592 (30%) were Gram positive cocci (GPC) and 4534 (74%) were Gram negative bacilli (GNB) (table-I).

Amongst 1592 Gram positive cocci 1198 (75%), 101 (6%) were MDR and XDR respectively. Frequency of MDR, XDR amongst GPC was shown in table-I. Coagulase positive *Staphylococci* were most frequently isolated GPC. *Enterococcus spp* exhibited highest resistance amongst GPC. No GPC was PDR.

Out of 4534 GNB 3481 (76.7%), 2620 (58%) and 72 (1.5%) were MDR, XDR and PDR respectively. Frequency of MDR, XDR and PDR amongst GNB was shown in table-II. The most

commonly isolated GNB was *E. coli*. Acinetobacter spp exhibited highest resistance.

Out of 6126 bacterial isolates 2855 (47%), 1885 (31%), 918 (15%) and 468 (7%) bacterial isolates were recovered from pus/tissue, urine, respiratory specimen and blood cultures respectively.

Out of 2855 bacterial isolates recovered from pus/tissue 2362 (82%) of isolates were MDR and 1096 (38%) were XDR. Coagulase positive *Staphylococciwer*e most frequently isolated pathogen accounting 1006 (35%) of isolates. *Acinetobacter spp* elicited highest resistance. Figure-1 shows that 88% and 0.6% of isolated Coagulase positive *Staphylococci*, 48% and 12% of isolated Enterococci, 84% and 70% of isolated *E.coli*, 88% and 59% of isolated Pseudomonas spp, 91% and 68% of isolated *Klebsiella spp* and 94% and 86% of isolated *Acinetobacter spp* from pus/tissue specimens were MDR and XDR respectively.

Out of 1885 bacterial isolates recovered from urine 1263 (67%), 945 (50%) and 15 (0.8%) were MDR, XDR and PDR respectively. *E. coli* was the most frequently isolated uropathogen accounting about 1342 (46%). *E.coli* and *Klebsiella spp* exhibited highest resistance. Fig-2 illustrates that 41% and 36% of isolated Enterococci from urine were MDR and XDR respectively. 72%, 59% and 0.7% of isolated *E.coli* and 70%, 16% and 2.1% of isolated *Klebsiella spp* from urine were MDR, XDR and PDR respectively. 48% and 40% of isolated Pseudomonas spp from urine were MDR and XDR respectively.

Table-I: Frequency of multi drug resistant, extensively drug resistant amongst gram positive cocci (n=1592).

Gram positive cocci	n	Multi drug resistant	Extensively drug resistant
Coagulase positive Staphylococci	1207 (76%)	1030 (85%)	6 (0.5%)
Enterococcus spp	385 (24.1%)	168 (44%)	95 (25%)

Table-II: Frequency of multi drug resistant, extensively drug resistant and pan drug resistant amongst gram negative bacilli (n=4534).

Gram Negative Bacilli	n	Multi drug resistant	Extensively drug resistant	Pan drug resistant
E. coli	2100 (46.3%)	1614 (76%)	1273 (60%)	11 (0.5%)
Pseudomonas aeruginosa	975 (21.5%)	648 (66%)	425 (43.5%)	14 (1.4%)
Klebsiella spp	779 (17.1%)	674 (86%)	434 (56%)	28 (4%)
Acinetobacter spp	423 (9.3%)	398 (94%)	371 (88%)	19 (4.4%)
Salmonella spp	43 (1%)	39 (91%)	30 (70%)	-
Others	214 (4.71%)	112 (60%)	76 (35%)	6 (2%)

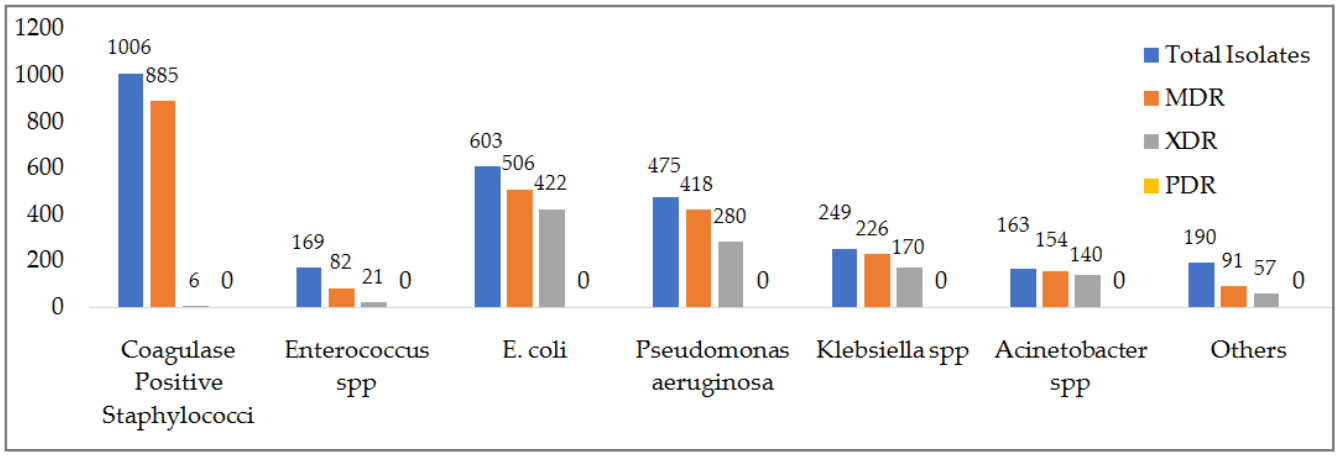

Figure-1: Pattern of multi drug resistant, extensively drug resistant and pan drug resistant bacterial isolates recovered from pus/ tissue specimen (n=2855).

Amongst 918 (32%) of isolated respiratory bacterial pathogens 661 (72%) were MDR, 483 (52%) were XDR and 41 (4.5%) were PDR. Pseudomonas aeruginosa was the most frequently isolated pathogen and highest resistance was exhibited by *Acinetobacter spp*. Figure-3 demonstrated that 36%, 24% and 2% of isolated Pseudomonas spp, 98%, 78% and 7% of isolated *Klebsiella spp*, 97%, 95% and 9% of isolated *Acinetobacter spp* and 90%, 35% and 1% of isolated *E.coli* from respiratory specimens were MDR, XDR and PDR respectively. About 60% of isolated *Staphylococcus spp* from respira-tory specimens were MDR.

Out of 468 (16.1%) isolated from Blood 393 (83%) were MDR, 197 (42%) were XDR and 16 (3.4%) bacterial isolates were PDR respectively. Coagulase positive *Staphylococci* were most frequently isolated pathogen accounting 130 (27%) of all isolated pathogen. 43 (9.1%) of blood cultures yielded growth of *Salmonella spp*. *Klebsiella spp* exhibited highest resistance. There were with about 78% of Coagulase positive *Staphylococci* isolated from blood were MDR. About 86%, 76% and 9% of isolated *Klebsiella spp* recovered from blood were MDR, XDR and PDR respectively. Almost 93% and 50% of isolated *E. colifrom* blood were MDR and XDR respectively. About 84% of isolated *Acinetobacter spp* from blood were MDR and 69% were XDR respectively. Amongst *Pseudomonas spp* isolated from blood 97%, 35% and 13% were MDR, XDR and PDR respectively. 100% of *Salmonella spp* isolated from blood were MDR and 70% were XDR respectively. 30% of

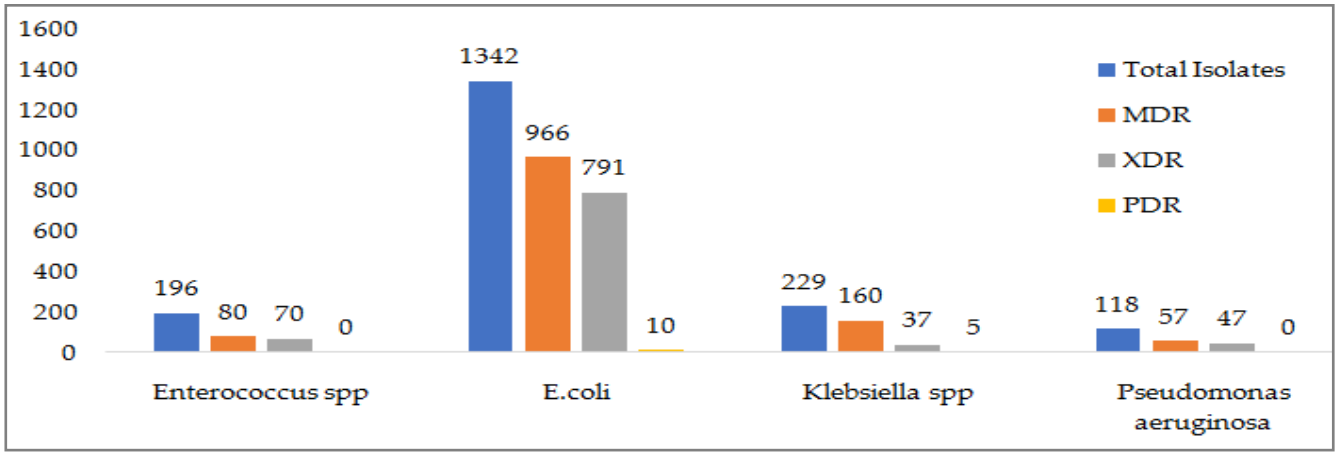

Figure-2: Pattern of multi drug resistant, extensively drug resistant and pan drug resistant bacterial isolates recovered from urine (n=1885).

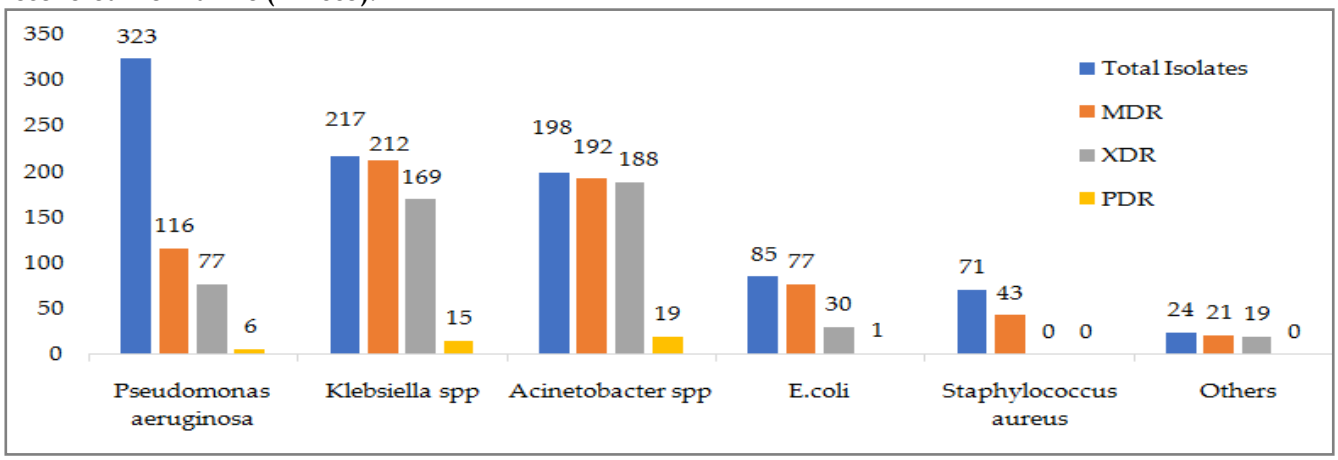

Figure-3: Pattern of multi drug resistant, extensively drug resistant and pan drug resistant bacterial isolates recovered from respiratory specimen (n=918).

isolated Enterococci from blood were MDR and 20% were XDR.

DISCUSSION

Resistance to antimicrobial agents is becoming pandemic phenomenon that can spread between regions and devoid of any International boundaries. This serious health care threat can drag the world into pre-antibiotic era[10]. Keeping in view the increase prevalence of these highly resistant superbugs throughout the world and lack of data especially from our part of the world such type of study is an urgent need in order to determine the burden of these highly drug resistant bugs in order to constitute proper infection control policies based on the antibiogram of our hospital.

In our study burden of MDR, XDR and PDR super bugs were found to be 76%, 44% and 1.2% respectively which was alarmingly high. Another study conducted at tertiary care hospital of Central India analyzed 1060 bacterial strains and reported comparatively low burden of MDR and XDR accounting about 37.7% and 13.8% respectively. No PDR was detected in that study[10]. A study conducted at tertiary care hospital in Abakiliki Metropolis reported almost similar percentage of MDR (70%)[13]. Out of 1592 GPC evaluated in our study 75% were MDR and 6% were XDR. No GPC was found to be PDR. Comparatively low prevalence of MDR (37%) GPC was reported in another study[10]. In our study *Enterococcus* exhibited highest resistance. Similar to our findings other studies also reported *Enterococcus* as an emerging Super bug and one of the Escape pathogens[13,14]. Out of 4534 GNB 76.7%, 58% and 1.5% were MDR, XDR and PDR respectively. *E.coli* was the most frequently isolated pathogen. Amongst GNB *Acinetobacter spps* how highest resistance. Similar results were obtained by another study carried out between January to June 2017 at Kalinga Institute of Medical Sciences (KIMS), a tertiary care teaching hospital in Bhubaneswar, Odisha[4]. A study carried out at tertiary care center in Pune, India reported comparatively low prevalence of MDR and XDR accounting about 49.6% and 15.1% respectively. Similar to our study *E. coli* was most commonly isolated GNR. In contrast to our study Pseudomonas aeruginosa exhibited highest resistance[1].

In our study 82% of isolates recovered from pus were MDR and 38% were XDR. Comparatively low prevalence of MDR (53.1%) and XDR (9.1%) bacteria was reported in another study conducted by Basak *et al*[10]. Coagulase positive *Staphylococci* was most frequently isolated pathogen. *Staphylococcus* has been reported as the predominant organism in other studies also[15]. The possible reasonfor the high frequency of this microorganism is that *Staphylococci* are present as normal flora on the skin, when there is a breach on skin and soft tissues, they get displaced to other sterile sites and disseminate easily. *Acinetobacter spp* elicited highest resistance with 94% and 86% of isolated spp were MDR and XDR respectively. Similar findings were reported by another study conducted by Basak *et al*[10]. Out of 64% of bact-erial isolates recovered from urine 67%, 50% and 0.8% were MDR, XDR and PDR respectively. Almost similar prevalence of MDR and XDR was reported in another study conducted in Dhaka city, Bangladesh[16]. *E.coli* was the most isolated uropathogen accounting 46% and also exhibited highest resistance. *E. coli* has been regarded as predominant uropathogen in other study conducted by Begum *et al* in 2015[17].

In our study out of 32% of bacteria isolated from respiratory samples 72% were MDR and 52% were XDR and 4.5% were PDR. Comparatively low prevalence of 56% MDR and 37% XDR recovered from tracheal isolates was reported by another study[18]. Pseudomonas aeruginosa was the most frequent isolated pathogen in our study similar to another study conducted in Shalamar Medical and Dental College Lahore[18]. Highest resistance was exhibited by *Acinetobacter spp* in our study with 97%, 95% and 10% of isolated *Acinetobacter spp* were MDR, XDR and PDR respectively. In comparison another study revealing pattern of antimicrobial resistance in clinical isolates of *Acinetobacter spp* at tertiary level health

care facility in Northern India reported low prevalence of MDR and XDR[19]. *Acinetobacter spp* that were once considered as opportunistic, low virulence pathogens have now emerged as an important nosocomial pathogen due to their ability to form biofilms and easy acquisition of drug resistance[20]. In our study 83%, 42% and 3.4% of bacteria isolated from blood were MDR, XDR and PDR respectively. Comparatively low prevalence of 28.7% MDR and 4.7% XDR was reported in another study conducted by Manchanda *et al*[21] Coagulase positive *Staphylococci* were most frequent isolated pathogen in our study accounting 27% of all isolated pathogen. Our finding was consistent with another study[22]. 9.1% of blood cultures in our study yielded growth of *Salmonella spp*. Out of these isolated 91% were MDR and 70% were XDR. Multiple studies supported our findings of very high prevalence of MDR isolates. Over 300 XDR typhoid cases has emerged in Pakistan since November 2016[23]. Continual emergence of anti-biotic resistance has led to treatment failures and makes treatment of enteric fever more chall-enging. A new conjugate vaccine approved in 2018 offers an important tool to control typhoid in South Asia. Investments in research, development of rapid diagnostic tests and new treatments are the need of hour[24,25].

High burden of MDR, XDR and PDR super bugs isolated from various indoor microbiological specimen at tertiary care centers of Rawalpindi observed in our study might be associated with lack of antibiotic resistance surveillance, irrational use of antimicrobials and poor infection control policies. Similar studies are required from other centers across Pakistan to exactly ascertain the magnitude of problem.

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RECOMMENDATION

Emergence of such bugs need to be curtailed. This situation warrants the implementation of an efficient infection control and antibiotic stewardship programs. Close surveillance of drug resistant bacteria is highly recommended to reduce the menace of antimicrobial resistance. Coordinated efforts to implement new policies, renew research efforts and investment into new antimicrobials are needed to manage drug resistant crises.

CONCLUSION

The burden of MDR, XDR and PDR among bacteria isolated from various indoor microbiological specimen was 76%, 44% and 1.2%, respectively, which is disturbingly high and highlights a serious public health problem. These findings are alarming because infections caused by these drug resistant bacteria are difficult to treat with currently available antibiotics. Such infections lead to higher morbidity, mortality and impose huge healthcare cost.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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