Acta Scientific MICROBIOLOGY (ISSN: 2581-3226)

Volume 5 Issue 6 June 2022

Research Article

Increasing Resistance to Most of the Commonly Used Antibiotics in Eastern India

Anindya Das[1]* and Diganta Dey[2]

[1]Assistant Professor, Department of Microbiology, KPC Medical College and Hospital, West Bengal University of Health Science, Kolkata, India

[2]Department of Microbiology, Ashok Laboratory Clinical Testing Centre Pvt. Ltd., Kolkata, India

***Corresponding Author:** Anindya Das, Assistant Professor, Department of Microbiology, KPC Medical College and Hospital, West Bengal University of Health Science, Kolkata, India.

DOI: 10.31080/ASMI.2022.05.1086

Received: April 25, 2022

Published: May 30, 2022

Abstract

Infectious diseases continue to be a global health problem. In view of this, present work was initiated with an epidemiological assessment of antibiotic resistance pattern in Eastern India. This study was done in two phases; first one in 2009 - 10, and after an interval of about eight years, second one in 2018 - 19. The statistics showed a marked increase of extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae (~ 32% in community acquired urinary tract infections or CA UTI, and ~41% in hospital acquired or HA UTI in our second phase of study 2018 - 19) in comparison to the former study (~ 18%; during 2009 - 10). By and large, these ESBL producing Enterobacteriaceae were found to exhibit greater resistance against several non-β-lactam antibiotics, as compared to ESBL non-producer counterparts. The 'phase 2' study also showed an alarming rise in carbapenem resistant Enterobacteriaceae (CRE) cases, with a frequency of ~ 10% in CA and ~ 20% in HA pathogens. Furthermore, an alarming rise in flouroquinolone resistance was noted among the Gram negative, as well as the Gram positive bacteria. However, the number of methicillin resistant *S. aureus* (MRSA) was found to be comparatively less (~25% in CA UTI and ~37% in HA UTI) during the 'phase 2' than in the 'phase1' study (~52%). Again, MRSA isolates exhibited significantly ($p < 0.05$) higher rates of resistance against β-lactam/β-lactamase inhibitor combinations and fluoroquinolones, as compared to the methicillin sensitive (MSSA) isolates. Overall, nosocomial organisms were more resistant against the tested antimicrobials than their community-acquired counterparts. The study demonstrated increasing resistance to most of the commonly used antibiotics, which mandates stringent antibiotic stewardship.

Keywords: Multi-drug Resistant Bacteria (MDR); Extended Spectrum Beta-lactamase Producing Bacteria (ESBL); Nosocomial Infection; Community Acquired Pneumonia; Methicillin Resistant *Staphylococcus aureus* (MRSA); Methicillin Sensitive *Staphylococcus aureus* (MSSA)

Introduction

'Drug resistance follows the drug like a faithful shadow'

Paul Ehrlich

The escalating phenomenon of antimicrobial resistance has already plunged the world into a post-antibiotic era, affirming the prophetic premonition of the Nobel Laureate, who passed away hundred years back, in August of 1915 [1]. The increasing frequency of drug resistance has been attributed to several reasons, such as, selection pressure due to indiscriminate use of antibiotics, intrinsic resistance of 'superbugs', and various societal factors that enhance the transmission of drug-resistant organisms [2-4]. At present, this is a huge concern for public health, which has been all the more exacerbated by a declining number of antimicrobials coming out of

the discovery pipeline in the recent past [5]. Eventually, the definition of 'multidrug resistant' (MDR) bacteria has evolved to classify a host of resistance patterns associated with the therapeutic failures occurring in numerous clinics around the world. Thus, the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC in Atlanta) jointly proposed that MDR would be the "acquired non-susceptibility to at least one agent in three or more antimicrobial categories" [6].

Antibacterial resistance has become one of the serious concerns, worldwide, in current millenium. It is reported that about 25,000 people in the E.U. and 63,000 in the U.S. die each year due to infections caused by multi-drug resistant (MDR) bacteria [7]. Penicillin- and macrolide-resistant *Streptococcus pneumoniae*, methicillin-resistant *Staphylococcus aureus* (MRSA) and multi-drug resistant (MDR) enteric pathogens cause the majority of community associated infections [8]. Furthermore, MRSA and vancomycin-resistant *S. aureus* and Enterococci, extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, and MDR non-fermenters are considered as the deadliest nosocomial pathogens around the world. New Delhi metallo-β-lactamase-1 (NDM-1) was found in almost every continent within a year of its emergence in India [9]. Multi-drug resistant (MDR) Gram-negative bacteria are posing a greater threat to public health, because their increase in resistance would be faster than the Gram-positive ones, and there are fewer new antibiotics under development to provide sufficient therapeutic coverage in the near future [10]. In view of this, present work was initiated with an epidemiological assessment of antibiotic resistance pattern to commonly used antibiotics in a reference laboratory in Kolkata, Eastern India.

Materials and Methods

Clinical isolates were taken from both the community and hospital acquired (CA and HA) infections which included patients suffering from superficial wounds, urinary tract, enteric and pulmonary infections, and bacteremia; and attending Ashok Laboratory Clinical Testing Centre Pvt. Ltd. and Ashok Laboratory Satellite Centre at MR Bangur Hospital, Kolkata during the study period covering the duration of July 2009 to February 2010 and after an interval of 8-9 years, from July 2018 to September 2019. Reference strains were procured from American Type Culture Collection (ATCC), USA. In addition, particular care was taken to avoid the issues of apparent *in vitro* susceptibility for any particular antibiotic, which often fails to detect the *in vivo* resistance due to presence of certain enzymatic inactivation processes, such as β-lactamase, ESBL, AmpC β-lactamase, MBL, etc. Hence, the selected Gram negative bacteria were specifically characterized in terms of these enzymes which are typically responsible for their clinical resistance. Further, the resistance to the penicillinase-stable penicillins were checked in *S. aureus* isolates (referred to as "methicillin resistant *Staphylococcus aureus*"/MRSA; Collee., *et al*. 1996; CLSI, 2015).

All the Gram-negative and Gram-positive bacteria were identified by their staining character, morphology, motility, growth and appearance in different selective media; and biochemical characteristics. Phenotypic screening was done for Methicillin resistance in *Staphylococcus aureus* and Gram negative bacteria were screened for ESBL-production by performing the following tests:

Initial screen test

Disc diffusion tests were performed to screen presence of ESBLs in *K. pneumoniae*, *E. coli* and *Proteus* spp. by using cefpodoxime (10 µg), ceftazidime (30 µg), aztreonam (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg) discs as recommended by the CLSI. In case of ESBL-producing *Klebsiella* and *E.coli*, the respective zone diameter was as follows: cefpodoxime ≤17 mm, ceftazidime ≤22 mm, aztreonam ≤27 mm, cefotaxime ≤27 mm and ceftriaxone ≤25 mm. This criteria was specifically altered for *Proteus* spp. as follows: cefpodoxime ≤22 mm, ceftazidime ≤22 mm and cefotaxime zone ≤27 mm (CLSI 2015).

Phenotypic confirmatory test

Disc diffusion tests were performed for phenotypic confirmation of the presence of ESBLs in *K. pneumoniae* and *E. coli* and *Proteus* spp. by using cefotaxime (30 µg) and ceftazidime discs (30 µg) with and without clavulanate (10 µg) as recommended by CLSI. In case of ESBL-producing bacteria, the zone diameter of cephalosporin/clavulanate disc will be at least 5mm greater than the zone for cephalosporin disc alone. *K. pneumoniae* ATCC 700603 was used as positive control for this test (CLSI 2015).

Double-disc synergy test

A third generation cephalosporin, namely ceftazidime or cefotaxime (30 µg), and a disc of co-amoxiclav (20 µg amoxicillin/10 µg clavulanic acid) were placed 20 mm apart on Mueller Hinton agar (MHA) plate on which 0.5 McFarland of test organism was

swabbed. In case of an ESBL producer strain, the zone diameter of cephalosporin disc was found to extend towards the co-amoxiclav disc (Figure 1; Collee., *et al*. 1996; Dey., *et al*. 2012).

Figure 1: (a) Double-disc synergy and (b) Phenotypic confirmatory test for ESBL detection. Antibiotic discs used for these procedures were (i) ceftazidime (30 µg), (ii) ceftazidime-clavulanic acid (30/10 µg), (iii) amoxy-clavulanic acid (20/10 µg), and (iv) cefotaxime (30 µg).

Methicillin resistance in *Staphylococcus aureus*

Staphylococcus aureus isolates were subjected to cefoxitin disc diffusion test using a 30 µg disc. A 0.5 Mc Farland standard suspension of the isolate was made and lawn culture done on MHA plate. Plates were incubated at 37 ^{0} C for 18 h and a zone diameter of ≤ 21 mm was reported as methicillin resistant (MRSA) and ≥22 mm was considered as methicillin sensitive (MSSA; Swenson., *et al*. 2001; CLSI 2015).

Antibacterial susceptibility studies were carried out by Kirby and Bauer disk diffusion technique using commercially available antibiotic discs (HiMedia, Mumbai, India). Bacterial culture in peptone water (Himedia, Mumbai, India), containing 0.5 McFarland turbidity (1 x 10^{8} cfu/mL), was swabbed in Mueller Hinton agar (MHA) plate.

Statistical analysis

The chi-square test was used to compare different groups. A p-value of less than 0.05 was considered as statistically significant. Statistical softwares GraphPad Prism (GraphPad Inc, San Diego, USA) and microsoft office Excel 2007 (Microsoft Corporation, USA) were used to prepare graphs and analyse the data.

Results

Phase 1 study (2009 -2010)

Associated bacteria

Prevalence of ESBL producers

A total of four hundred isolates, comprising of *E. coli* (two hundred) and *K. pneumoniae* (two hundred) were checked for ESBL production by performing double-disc synergy test and phenotypic confirmatory test. Figure 2 showed the percentage of ESBL producing organisms found for each of the aforesaid genera.

Figure 2: Percentage of ESBL producing organism in (A) *Escherichia coli* and (B) *Klebsiella pneumoniae.*

Prevalence of MRSA

Two hundred and forty one *Staphylococcus aureus* isolates from the clinic were screened for methicillin resistance by cefoxitin disc diffusion test. Figure 3 depicted the prevalence of MRSA in CA infections of *S. aureus.*

Figure 3: Percentage of MRSA obtained by screening of *Staphylococcus aureus* clinical isolates (n = 241).

Distribution of ESBL producing bacteria

A total of two hundred ESBLs-producing isolates, comprising of *E. coli* (138), *Klebsiella* spp. (49) and *Proteus* spp. (13), were obtained from clinical specimen of the patients from various age groups. Overall, ESBLs-producing enteric Gram negative rods (EGNRs) were most prevalent in older age, for example, 51-60 years age group (36 out of 200; 18%), 61-70 years age group (38 out of 200; 19%), and 71-80 years age group (28 out of 200; 14%). Figure 4 elaborated the prevalence of ESBL-producing EGNRs' infection found in patients of different age groups and gender.

Figure 4: Prevalence of ESBL-producing organisms associated infections in different age groups of male and female patients.

Distribution of MRSA

The highest number of MRSA infection (14; 12%) was found in male patients of 51 - 60 years age group. In female, maximum MRSA infection (~10; 8.5%) occurred in the age group 21 - 30 years and 51 - 60 years. Overall, two distinct peaks were observed in the age group of 20-40 years and 50-80 years (Figure 5).

Figure 5: Prevalence of MRSA infections in different age groups of male and female patients.

Antibiotic resistance

Fluoroquinolone resistance and ESBL: a correlation study

Results of this study showed ESBL producing isolates were significantly resistant ($p < 0.05$) to ciprofloxacin as compared to their ESBL non-producing counterparts. It was also concluded that such relationship was statistically non-significant for five other fluoroquinolone antibiotics (Figure 6). Incidentally, clinical application of gatifloxacin, included in this study, has been discontinued in India since March 2011 (The gadget of India notification, 2011).

Figure 6: Fluoroquinolone resistance observed among four hundred clinical isolates of ESBL producer and ESBL non-producer bacteria: (A) *Escherichia coli* (n = 200) and (B) *Klebsiella pneumoniae* (n = 200). Significantly different resistance pattern (*) between ESBL producer and ESBL non-producer isolates was obtained by chi-square test ($p < 0.05$).

MRSA vs. MSSA: antibiotic resistance

Present study showed *S. aureus* clinical isolates were potentially resistant to ampicillin, cefixime, ciprofloxacin and sparfloxacin. Vancomycin and cefoperazon/sulbactum were the most effective antibiotics against these isolates. However, MRSA isolates were selectively resistant ($p < 0.05$) against some antibiotics, viz. cefuroxime, ceftazidime, doxycycline, norfloxacin and ofloxacin, as compared to MSSA isolates (Figure 7).

Phase 2 study (2018 - 2019)

Associated bacteria

Significant bacteriuria

A large number (1799) of urine samples were obtained from community patients coming to the clinic. Significant bacteriuria was detected in 587 out of 1799 cases (~ 32%). In contrast, pa-

Figure 7: Antibiotic resistance patterns of two forty-one *S. aureus* isolates, comprising of methicillin resistant (MRSA; n = 126) and sensitive (MSSA; n = 115) *S. aureus* isolates.
Significantly different resistance pattern (*) between MRSA and MSSA was obtained by chi-square test ($p < 0.05$).

tients suffering from nosocomial infection, 287 out of 704 (40%) specimens were found to be culture positive (Figure 8). Thus, significantly ($p < 0.001$) higher bacteriuria was found in HA infections as compared to CA urinary tract infection. However, polymicrobial infection was not detected among the CA UTI cases, whereas, five HA UTI cases were found with polymicrobial infection [11].

Figure 8: Comparison between the occurrence of significant bacteriuria in CA and HA UTI.

Isolated uropathogens

E. coli and *Klebsiella* spp. were the major uropathogens isolated from CA and HA UTI. While *E. coli* were more predominant among CA cases (44%), *K. pneumoniae* infections were found in comparatively greater number (39%) of HA UTI cases. Further, *S. saprophyticus* (6%) in CA and Enterococci (10%) in HA were found to be the most prevalent Gram-positive organisms (Figure 9).

Figure 9: Comparison between uropathogens isolated from CA and HA UTI.

ESBL producing Enterobacteriaceae: prevalence and distribution

Percentage of ESBL producers, as shown in figure 10 A, were found to be greater among HA infections (41.4%) as compared to CA infection (31.8%). ESBL producing *Klebsiella* isolates were more prevalent in HA infection (48%) in comparison to CA UTI (34.5%; Figure 10 B).

Carbapenem resistance

Resistance to carbapenem drugs (e.g. meropenem, imipenem) was found to be double in HA isolates (20%) as compared to CA pathogens (10%). Again, among the CA pathogens, mainly *Pseudomonas* spp. (16%) and *Acinetobacter* spp. (15%) were associated with carbapenem resistant infection, whereas about 9% of Entero-

Figure 10: (A) Prevalence and (B) types of ESBL producing Enterobacteriaceae isolated in CA and HA UTI.

bacteriaceae were found to develop resistance to carbapenems. In comparison, carbapenem resistance was much greater (20%) among the Enterobacteriaceae among the nosocomial infections, while 27% of *Pseudomonas* spp. exhibited resistance to carbapenem (Figure 11).

Figure 11: Prevalence of carbapenem resistance in (A) CA and (B) HA uropathogens.

Distribution of Gram-positive cocci

While Enterococci were prevalent (52%) among Gram-positive cocci (GPC) obtained from HA UTI, *Staphylococcus saprophyticus* predominated (62%) in CA infection (Figure 12). Further, the percentage of MRSA was found to be greater (~37%) in HA UTI as compared to CA infection (25%).

Figure 12: Distribution of Gram-positive cocci (GPC) in CA and HA infections.

Antibiotic resistance

Enterobacteriaceae vs. non-fermenters: antibiotic resistance

From the data displayed in figure 13, imipenem, meropenem and amikacin were found to be the most susceptible antibiotics against Enterobacteriaceae. Amoxicillin/clavulanic acid and piperacillin/tazobactam were also effective among the β-lactam/β-lactamase inhibitor groups of antibiotics. All fluoroquinolones, except levofloxacin, exhibited higher resistance trend against these isolates. Again, all non-fermenters were found to be sensitive to colistin. However, more than 40% of non-fermenters exhibited resistance to several antibiotics, such as, piperacillin, aztreonam, cefipime, ceftazidime, norfloxacin and ofloxacin (Figure 13B).

Figure 13: Comparative resistance trends in (A) Enterobacteriaceae and (B) non-fermenter Gram-negative bacilli isolated from CA and HA UTI.

ESBL producer vs. non-producer: resistance to other antibiotics

Since ESBLs are resistant to third- and fourth-generation cephalosporins and monobactams, therefore, we studied the comparative resistance pattern of ESBL producer and ESBL non-producer bacteria against other antibiotics, such as fluoroquinolones, amikacin, gentamicin, cotrimoxazole, tetracycline and nitrofurantoin. The result in figure 14(A) indicated that ESBL producers, in general, exhibited greater resistance to these antibiotics in comparison to their non-ESBL counterparts. However, the trend was independent of the source (CA or HA) of infection, which indicated that ESBL production was the major determinant for developing resistance against these groups of antibiotics (Figure 14B).

Figure 14: (A) Comparative resistance patterns between ESBL producer and non-producer organisms against other groups of antibiotics. (B) Similar resistance trends were observed between CA and HA uropathogens of ESBL producer/ non-producer.

Staphylococcus vs. *Enterococcus*: antibiotic resistance

All Gram-positive uropathogens exhibited sensitivity to vancomycin and linezolid. However, a greater percentage of cefoxitin resistance among HA uropathogens was observed (Figure 15 A), indicating that these infections were associated with MRSA in particular. Again, ciprofloxacin, levofloxacin, norfloxacin, nitrofurantoin and tetracycline were found to be moderately sensitive (< 40% resistance) against *S. aureus* isolates, although the same drugs exhibited comparatively higher resistance (>50%) against *Enterococcus* spp. (Figure 15).

Figure 15: Comparative resistance pattern of (A) *S. aureus* and (B) *Enterococcus* spp. associated in CA and HA UTI.

Summary: Phase 2 study

A brief summary of the findings from phase 2 resistance study was given in table 1, which showed that HA UTI could be characterized by greater prevalence of Gram-negative bacilli, ESBL producer organisms and MRSA, in comparison to CA UTI. Again, the proportion of carbapenem and fluoroquinolone resistant isolates were greater among HA uropathogen as compared to CA isolates (Table 1).

	CA UTI	HA UTI
Significant bacteriuria	32%	41%
GNB[1] : GPC[2]	91: 9	80: 20
Most common GNB[1]	*E. coli*	*Klebsiella* spp.
Most common GPC[2]	*S. saprophyticus*	*Enterococcus* spp.
ESBL[3] producers	31.81%	41.41%
Carbapenem resistance	10%	20%

CRE[4]	9.09%	19.69%
Flouroquinolone resistance (except levofloxacin) for *S. aureus*	25% -37%	37% - 42%
Flouroquinolone resistance (except levofloxacin) for *Enterococcus* spp.	66%-68%	68%-72%
Levofloxacin resistance for GNB[1]	18% -21%	23%- 27%
Levofloxacin resistance for *S. aureus*	0%	21%
Levofloxacin resistance for *Enterococcus* spp.	58%	62%
MRSA[5]	25%	36.84%

Table 1: Comparative statistics of CA and HA UTI.

[1]Gram-negative bacilli; [2]Gram-positive cocci; [3]extended spectrum beta-lactamase; [4]carbapenem-resistant Enterobacteriaceae; [5]Methicillin-resistant *Staphylococcus aureus.*

Discussion:

Epidemiology of antibacterial resistance

Phase 1 study (2009 - 10)

The epidemiology of antibiotic resistance can exhibit remarkable geographical variability and rapid evolution over the time, due to a complex interplay of factors involved in selection and spread of different resistant bacteria and resistance genes, which have been partially understood so far [12]. Information about antibiotic-resistant bacteria causing diseases in community is sparse in Eastern India. Yet, such knowledge can be used to choose an optimal treatment procedure, in order to minimize the emergence and to plan for an effective infection control-strategy [13].

Therefore, we initially aimed to analyse the burden, distribution and antibiotic resistance pattern of some clinically significant bacteria obtained from community patients during July 2009 to February 2010 (Section 4.1). In this study, about 15.5% and 21% of incidents of ESBL-producing *E. coli* and *K. pneumoniae*, respectively, were noted (Figure 2). The observation could be compared with contemporary studies on percentage of ESBL-producing Enterobacteriaceae reported from other countries, such as, North America (3%), Western Europe (6%), Latin America (6%), Asia Pacific (8.6%), Eastern Europe (10%), Brazil (17.3%), Tanzania (24.4%), and Pakistan (60%) [14-17]. Again, in our study, a higher frequency of MRSA (52%; Figure 3) was observed among the Staphylococcal isolates. In fact, our observation was more or less comparable to the reports on prevalence of MRSA in some other countries [18,19]. Although, several surveys have been carried out on the prevalence of MRSA in Pakistan (22.9%) [20]; Iran (35.3%) [21]; Karachi (43%) [18]; and Lahore (63.64%) [19]; a high rate of CA-MRSA (85%) was reported from an urban community in central North Carolina, USA [22].

Both the ESBL- and MRSA- associated community infection was observed to be greater among the adults and elderly group of patients (Figure 4 and 5). Furthermore, ESBL-producing bacteria exhibited significantly higher rates of resistance against ciprofloxacin, norfloxacin, ofloxacin and sparfloxacin as compared to their non- ESBL- producer counterparts. In fact, genetic characterization of ESBL strains revealed that CTX-M β-lactamases were associated with nalidixic acid and fluoroquinolone resistance, and in some cases, this association was linked to the plasmid mediated quinolone resistance determinant [23]. An increased resistance against all tested fluoroquinolones was observed in Enterobacteriaceae, except in a couple of third generation fluoroquinolone antibiotics (Levofloxacin and Gatifloxacin; Figure 6). In fact, prolonged use of second generation quinolones is identified as one of the main reasons for development of such a high level of resistance forms against these fluoroquinolone drugs in the community [24]. Similarly, in our laboratory, the MRSA isolates exhibited significantly ($p < 0.05$) higher rates of resistance against ampicillin/sulbactum, amoxicillin/clavulanic acid, cefuroxime, doxycycline, norfloxacin, ofloxacin, piperacillin/tazobactum, ceftazidime, cefixime and cefoperazone/sulbactum, as compared to their MSSA counterparts. Increased resistance in *S. aureus* was observed against cefixime (41.3%), ciprofloxacin (31.6), ampicillin/sulbactum (27.8%) and norfloxacin (27.2%). Only 2.4% of isolated MRSA were found to be resistant to vancomycin, a glycopeptide agent (Figure 7). Similar findings were observed by many other authors [22,25-27]. Therefore, it appears that vancomycin could be the drug of choice for treatment of life threatening infection caused by multidrug resistant MRSA, at present.

Phase 2 study (2018 - 19)

Phase 2 study was conducted during the period July 2018 - September 2019, to evaluate the trend of antimicrobial resistance among isolates of urinary tract infection, both 'community acquired', as well as nosocomial origin the latter having been identified according to the CDC classification [28]. Until recent years, the majority of resistant pathogens were believed to be associated with nosocomial infections [29], although the recent data suggested the emergence of resistant microorganisms in community acquired infections also [30-33]. During the late 1990s and 2000s, Enterobacteriaceae (mostly *Escherichia coli)* had been identified to produce extended spectrum β-lactamase (ESBL), causing the majority of UTI cases in the community [34].

In our study, nosocomial organisms were more resistant to the tested antimicrobials, as compared to their community-acquired counterparts. The observation was in agreement with epidemiological studies conducted elsewhere [31]; [,35]. Interestingly, there was a marked increase of ESBL producing Enterobacteriaceae (~ 32% in CA UTI and ~41% in HA UTI; Figure 10A) in 'phase 2' study (2018 - 19) in comparison to 'phase 1' study (~ 15% of *E. coli* and 21% of *K. pneumoniae*) conducted 9 years ago (2009 - 10). A similar observation was reported from our neighboring country Pakistan where rapid increase in incidence of ESBL producing *E. coli* was noted from the year 2005 (33.7%) to 2009-10 (60.0%) [17]. In our study, ESBL producing Enterobacteriaceae exhibited overall greater resistance for other antibiotics, such as fluoroquinolones, amikacin, gentamicin, cotrimoxazole, tetracycline and nitrofurantoin, as compared to non-ESBL producers (Figure 14). Furthermore, an alarming rise in flouroquinolone resistance (except levofloxacin) was noted among the Gram negative bacteria, as well as *Staphylococcus aureus* (Figure 13 and 15A). Even, levofloxacin resistance was found to be remarkably higher (Figure 15B) in *Enterococcus* spp. Although, the number of MRSA was found to be ~25% in CA UTI and ~37% in HA UTI in 'phase 2' study (Figure 12) and the prevalence was ~52% in our 'phase 1' study. These figures were not surprising as the prevalence of MRSA infections have decreased worldwide in recent years [36]. Recent data from the US also showed a reduction of 31% in MRSA infections for primary sepsis over a period, from 2005-2011 (CDC, 2013). In the United Kingdom, where MRSA bacteremia had been a notifiable disease for a long time, the drop in rates has been even more dramatic. The number of cases of MRSA bacteremia declined from 2935 in 2008/2009 to 924 in 2011/2012 (Public Health England, 2013). In French hospitals, the rate of MRSA infections dropped by 35% between 1993 and 2007, in the Paris region [36]. In most countries in the European Union, the proportion of MRSA among invasive *S. aureus* infections has been controlled significantly [37].

Recently, carbapenem resistant Enterobacteriaceae (CRE) infections posed a serious threat to public health due to high mortality rates, drug resistance creating limited treatment options, and the potential for widespread dissemination. Mortality rates of 40% to 50% on the average had been reported [38-40]. A surveillance report of CDC, Atlanta, USA, concluded that at least one CRE healthcare-associated infection had occurred in 4.6% of acute-care hospitals during 2012 (CDC, 2013). According to the Meropenem Yearly Susceptibility Test Information Collection Program, meropenem-resistant *Klebsiella pneumoniae* increased from 0.6% in 2004 to 5.6% in 2008 [41]. Carbapenem resistance has been reported in up to 4.0% of *Escherichia coli* and 10.8% of *K pneumoniae* isolates according to the reports of National Healthcare Safety Network [42]. Besides the common trend and resistance level, geographic variation was also exhibited in some regions. A report in the United Arab Emirates showed the rates of resistance to imipenem in *E. coli* and *Klebsiella* spp. were 35.7% and 29.8%, respectively [43], and much higher than the average rates. Our study also showed an alarming rise in CRE cases, with a frequency of ~ 10% in CA and ~ 20% in HA pathogens (Figure 11). Table 1 summarized some salient epidemiological differences between community acquired and nosocomial urinary tract infections.

Conclusion

This study, investigating the bacterial resistance to commonly used antibiotics was performed in two phases, one in 2009 - 10 (phase- 1), and after an interval of about eight years, the second one in 2018 - 19 (phase- 2). The result showed a marked increase in ESBL producing Enterobacteriaceae in both community acquired and hospital acquired infections and these ESBL producing Enterobacteriaceae were also found to exhibit greater resistance against several non-β-lactam antibiotics, as compared to ESBL non-producer counterparts. Furthermore, an alarming rise in flouroquinolone resistance was noted among both Gram negative, as well as Gram positive bacteria. The 'phase 2' study also showed an alarming rise in carbapenem resistant Enterobacteriaceae (CRE) cases. However, the number of methicillin resistant *S. aureus* (MRSA)

was found to be comparatively less during the 'phase 2' than in the 'phase 1' study. Therefore, we discourage the indiscriminate use of antibiotics and recommend careful empirical prescription, culture and antibiotic sensitivity testing and selection of drugs accordingly.

Bibliography

1. Wax RG., *et al*. "Bacterial Resistance to Antimicrobials". 2nd ed.. CRC Press, Taylor and Francis: Boca Raton, FL 33487-2742 (2008).

2. Alanis AD., *et al*. "Antibacterial properties of some plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders". *Journal of Ethnopharmacology* 100 (2005): 153-157.

3. Livermore DM. "Has the era of untreatable infections arrived?" *Journal of Antimicrobe and Chemotherapy* 64 (2009): i29-36.

4. Livermore DM. "Epidemiology of antibiotic resistance". *Intensive Care Medicine* 26 (2000): 14-21.

5. Högberg LD., *et al*. "The global need for effective antibiotics: challenges and recent advances". *Trends in Pharmacological Sciences* 31 (2010): 509-515.

6. Magiorakos AP., *et al*. "Multidrug-resistant, extensively drug-resistant and pan drug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance". *Clinical Microbiology and Infection* 18 (2012): 268-281.

7. Aminov RI. "A brief history of the antibiotic era: lessons learned and challenges for the future". *Frontiers in Microbiology* 1 (2010): 134.

8. Davies J and Davies D. "Origins and Evolution of Antibiotic Resistance". *Microbiology and Molecular Biology Reviews* 74 (2010): 417-433.

9. Kang C-I and Song J-H. "Antimicrobial resistance in Asia: current epidemiology and clinical implications". *Infectious Chemotherapy* 45 (2013): 22-31.

10. Kumarasamy KK., *et al*. "Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study". *Lancet Infectious Disease* 10 (2010): 597-602.

11. Brogden KA., *et al*. "Human polymicrobial infections". *Lancet* 365 (2005): 253-255.

12. Gould IM. "The epidemiology of antibiotic resistance". *International Journal of Antimicrobial Agents* 32 (2008): S2-9.

13. Avorn JL., *et al*. "Antibiotic resistance: synthesis of recommendations by expert policy groups Alliance for the Prudent Use of Antibiotics". WHO: Geneva Switzerland (2001).

14. Jones CH., *et al*. "Characterization and sequence analysis of extended-spectrum β-lactamase- encoding genes from *Escherichia coli, Klebsiella pneumoniae*, and *Proteus mirabilis* isolates collected during tigecycline phase 3 clinical trials". *Antimicrobial Agents and Chemotherapy* 53 (2009): 465-475.

15. Mshana SE., *et al*. "Prevalence of multiresistant Gram-negative organisms in a tertiary hospital in Mwanza, Tanzania". *BMC Research Notes* 2 (2009): 49.

16. Abreu AG., *et al*. "Nosocomial infection and characterization of extended spectrum β-lactamases-producing Enterobacteriaceae in Northeast Brazil". *Revista da Sociedade Brasileira de Medicina Tropical* 44 (2011): 441-446.

17. Habeeb MA., *et al*. "Rapid emergence of ESBL producers in *E. coli* causing urinary and wound infections in Pakistan". *Pakistan Journal of Medical Sciences* 29 (2013): 540-544.

18. Saima P., *et al*. "Antimicrobial susceptibility pattern of clinical isolates of methicillin resistant *staphylococcus aureus". Journal of Pakistan Medical Association* 2 (2007): 57.

19. Iffat C., *et al*. "Sensitivity patterns of staphylococcus aureus isolates from services hospital Lahore". *Pakistan Postgraduate Medical Journal* 13 (2002): 170-173.

20. Akhter R., *et al*. "Isolation and antimicrobial susceptibility pattern of methicillin resistant and methicillin sensitive *Staphylococcus aureus". Journal of Surgery Pakistan* 14 (2009): 161-165.

21. Aghazadeh M., *et al*. "Sensitivity pattern of methicillin resistance and methicillin sensitive *Staphylococcus aureus* isolates, against several antibiotics including trigecycline in Iran. A hospital based study". *Pakistan Journal of Medical Sciences* 25 (2003): 443-446.

22. Magilner D., *et al*. "The prevalence of community-acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) in skin abscesses presenting to the pediatric emergency department". *MC Medical Journal* 69 (2008): 351-354.

23. Poirel L., *et al*. "Association of plasmid-mediated quinolone resistance with extended-spectrum β-Lactamase VEB-1". *Antimicrobial Agents and Chemotherapy* 49 (2005): 3091-3094.

24. King DE., *et al*. "New classification and update on the quinolone antibiotics". *American Family Physician* 61 (2000): 2741-2748.

25. Ahmed S. "Methicillin resistance among clinical isolates of staphylococcus aureus isolated at a microbiology diagnostic center in Kashmir". *Rawal Medical Journal* 34 (2009): 1.

26. Tahnkiwale SS., *et al*. "Methicillin resistance among isolates of *Staphylococcus aureus*: antibiotic sensitivity pattern and phase typing". *Indian Journal of Medical Sciences* 56 (2002): 330-334.

27. Qureshi AH., *et al*. "The current susceptibility pattern of methicillin resistant *Staphylococcus aureus* to conventional anti Staphylococcus antimicrobials at Rawalpindi". *Pakistan Journal of Medical Sciences* 20 (2004): 361-364.

28. Garner JS., *et al*. "CDC definitions for nosocomial infections". *American Journal of Infection Control* 16 (1988): 128-140.

29. Zaman R and Dibb WL. "Methicillin resistant *Staphylococcus aureus* isolated in Saudi Arabia: Epidemiology and antimicrobial resistance patterns". *Journal of Hospital Infection* 26 (1994): 297-300.

30. Borer A., *et al*. "Extended-spectrum beta-lactamase-producing Enterobacteriaceae strains in community-acquired bacteremia in Southern Israel". *Medical Science Monitor* 8 (2002): CR44-47.

31. Sturenburg E and Mack D. "Extended-spectrum β-lactamases: Implications for the clinical microbiology laboratory, therapy, and infection control". *Journal of Infection* 47 (2003): 273-295.

32. Rodríguez-Baño J., *et al*. "Epidemiology and clinical features of infections caused by extended-spectrum beta-lactamase producing *Escherichia coli* in nonhospitalized patients". *Journal of Clinical Microbiology* 42 (2004): 1089-1094.

33. Valverde A., *et al*. "Dramatic increase in prevalence of fecal carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae during nonoutbreak situations in Spain". *Journal of Clinical Microbiology* 42 (2004): 4769-4775.

34. Pitout JD., *et al*. "Emergence of Enterobacteriaceae producing extended-spectrum beta-lactamases (ESBLs) in the community". *Journal of Antimicrobe and Chemotherapy* 56 (2005): 52-59.

35. Guembe M., *et al*. "Evolution of antimicrobial susceptibility patterns of aerobic and facultative gram-negative bacilli causing intra-abdominal infections: results from the SMART studies 2003-2007". *Revista Española de Quimioterapia* 21 (2008): 166-173.

36. Jarlier V., *et al*. "Curbing methicillin- resistant Staphylococcus aureus in 38 French hospitals through a 15-year institutional control program". *Archives of Internal Medicine* 170 (2010): 552.

37. Meyer E., *et al*. "The reduction of nosocomial MRSA infection in Germany: An analysis of data from the hospital infection surveillance system (KISS) between 2007 and 2012". *Deutsches **Ärzteblatt** International* 111 (2014): 331-336.

38. Patel G., *et al*. "Outcomes of carbapenem-resistant *Klebsiella pneumoniae* infection and the impact of antimicrobial and adjunctive therapies". *Infection Control and Hospital Epidemiology* 29 (2008): 1099-1106.

39. Schwaber MJ., *et al*. "Containment of a countrywide outbreak of carbapenem-resistant *Klebsiella pneumoniae* in Israeli hospitals via a nationally implemented intervention". *Clinical Infectious Diseases* 52 (2011): 848-855.

40. Chitnis A., *et al*. "Outbreak of carbapenem-resistant Enterobacteriaceae at a long-term acute care hospital: sustained reductions in transmission through active surveillance and targeted interventions". *Infection Control and Hospital Epidemiology* 33 (2012): 984-992.

41. Rhomberg PR and Jones RN. "Summary trends for the Meropenem Yearly Susceptibility Test Information Collection Program: a 10-year experience in the United States (1999-2008)". *Diagnostic Microbiology and Infectious Disease* 65 (2009): 414-426.

42. Hidron AI., *et al*. "NHSN annual update: antimicrobial resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007". *Infection Control and Hospital Epidemiology* 29 (2008): 996-1011.

43. Al-Dhaheri AS., *et al*. "Resistance patterns of bacterial isolates to antimicrobials from 3 hospitals in the United Arab Emirates". *Saudi Medical Journal* 30 (2009): 618-623.