Trends in antimicrobial resistance amongst pathogens isolated from blood and cerebrospinal fluid cultures in Pakistan (2011-2015): a retrospective cross-sectional study

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Nida Javaid
Lahore University of Management Sciences Syed Babar Ali School of Science and Engineering
ORCiD: https://orcid.org/0000-0002-8106-916X

Qamar Sultana
Department of Microbiology, Chughtai Laboratory

Karam Rasool
Department of Microbiology, Chughtai Laboratory

Sumanth Gandra
Washington University in Saint Louis School of Medicine

Fayyaz Ahmad
Department of Statistics, University of Gujrat

Safee Ullah Chaudhary
Lahore University of Management Sciences Syed Babar Ali School of Science and Engineering

Shaper Mirza
shaper.mirza@lums.edu.pk Corresponding Author
ORCiD: https://orcid.org/0000-0002-6651-6101

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Abstract

**Background:** Only a few studies have presented long-term trends in antimicrobial resistance (AMR) at a nationwide level in Pakistan. This study presents a comprehensive situational analysis of AMR trends among pathogens isolated from blood and cerebrospinal fluid (CSF) cultures, between 2011 and 2015, in Pakistan.

**Methods:** A retrospective analysis of AMR data on pathogens isolated from Blood and CSF over a five year period was carried out. Susceptibility data on these pathogens was obtained from Chughtai Laboratory (CL). Then, proportion of the resistant pathogens were calculated and analyzed.

**Results:** Our results show that highest resistance rates against all tested antimicrobials were observed in *Acinetobacter* species. We observed a steep rise in carbapenem resistant *Acinetobacter* species from 50 to 95.5% between 2011 and 2015. Our results also highlight the emergence of third and fourth generation cephalosporins resistance in *Salmonella enterica* serovar Typhi in Pakistan. While we observed a rise in AMR in other major pathogens, we unexpectedly found decreasing resistance trends in *Staphylococcus aureus*.

**Conclusions:** Taken together, our results show an overall increase in AMR in pathogens isolated from blood and CSF cultures in Pakistan between 2011 and 2015.

**Background**

Invasive infections, defined as bloodstream and cerebrospinal fluid (CSF) infections, account for 5.3 million deaths around the world annually (1). The use of broad-spectrum antimicrobials for treatment of these infections has resulted in an increase in antimicrobial resistance (AMR). As a consequence, the treatment of such infections is becoming increasingly difficult leading to treatment failures and increased mortality. Availability of over the counter drugs in developing countries such as Pakistan has led to the epidemic of AMR in these countries. Indiscriminate usage of antimicrobials exerts an increased selection pressure on the bacterial population resulting in accelerated emergence of AMR (2). While developing countries are battling an accelerated spread of AMR, developed countries are also experiencing the same trend. In the United States between 1999 and 2012, 47.9% *Acinetobacter baumanii* were carbapenem resistant, 68.4% *Staphylococcus epidermidis* were
ciprofloxacin resistant, and 13.7% Escherichia coli (E. coli) were β-lactam resistant (3-5). Furthermore, a similar resistance landscape had emerged in Canada between 2007 and 2011, where 27% of E.coli were resistant to ciprofloxacin, 19.3% Staphylococcus aureus (S. aureus) were resistant to methicillin, and 16.8% Streptococcus pneumoniae (S. pneumoniae) were resistant to penicillin (6). Unavailability of reliable data in the developing countries like Pakistan, makes it difficult to monitor and develop insights into emergence of AMR (2). The limited number of studies undertaken to investigate resistance in Pakistan indicate that most of the pathogens are resistant to commonly used antibiotics. For instance, between 1997 and 2014, 91% of E. coli were reported to be resistant to amikacin, while 91.7% Salmonella enterica serovar Typhi (S. Typhi) were fluoroquinolone resistant, and 90.9% Acinetobacter species were imipenem resistant (7-9). These studies indicate that AMR poses a burgeoning public health problem in Pakistan and highlights the need of an in-depth assessment of AMR situation.

Towards this goal, the present study examined AMR data from a large diagnostic lab to identify (i) the most prevalent pathogens isolated from blood and CSF cultures in Pakistan, (ii) the patterns of resistance amongst these pathogens, and (iii) their co-resistance trends.

Methods

For this retrospective cross-sectional study, AMR data collected on pathogens isolated from blood and CSF cultures over five years (2011-2015) were analyzed. Data on pathogens isolated and their antimicrobial susceptibility testing were obtained from Chughtai Lab (CL), a diagnostic facility with over 180 collection centers in 60 cities of Pakistan. Standard biochemical tests and Analytical Profile Index (API) identification kits (BioMerieux) (10) were used to identify bacterial species at CL. Antimicrobial susceptibility testing was done using disc diffusion method following Clinical Laboratory & Standards Institute (CLSI) guidelines (CLSI 2013).

Data on positive cultures were obtained from an electronic database containing patients’ reports. Information obtained on each case included: (1) patient’s age, (2) patient’s sex, (3) year of sample collection, (4) specimen source, (5) city in which the sample was drawn, (6) isolated pathogen in the positive culture, and (7) susceptibility results (defined as susceptible, intermediate, or resistant). All
cultures were examined without taking into consideration their clinical relevance. Of 3092 cases, datasets with missing information on age (n = 17) and sex (n = 2) were excluded from the study. Cases with information on pathogens isolated from sites other than blood and CSF (n = 5) were also excluded from the dataset. Most common microorganisms isolated from blood and CSF cultures were identified for further analysis (Fig. 1). Microorganisms were stratified by year of sample collection and patients’ age(< 5 years, 6–18 years, 19–45 years, 46–65 years, and 65 < years), and patients’ sex. For each case, intermediate resistance was considered as resistant. Susceptibility for all tested antimicrobials was examined and reported in each species separately. The bacterial species analyzed included Coagulase-negative staphylococci (CoNS), E. coli, Acinetobacter species, S. aureus, and S. Typhi.

Statistical analysis
Statistical analyses were performed using IBM SPSS statistics 22 (SPSS Inc., Chicago, Ill., USA). SAS university edition software (Cary, NC: SAS Institute Inc) was employed to perform Cochran Armitage test for trends. Figures were plotted using Circa software (www.omgenomics.com). P-value of < 0.05 was considered significant for all statistical analyses. Distribution of cases as well as the pathogens over patients’ age, patients’ sex, site of infection, and year of isolation was calculated using univariate analysis. Unadjusted resistance rates in these pathogens was determined over age groups, sex, and year of isolation using univariate analysis. As the number of isolates varied annually throughout the study period, proportions of the resistant pathogens were evaluated to normalize the data. The proportion of resistant isolates were examined over age and sex respectively using Fisher’s exact test, with Bonferroni corrections. Cochran Armitage test for trends was used to investigate trends of AMR over time. Pairwise resistance (referred to as co-resistance) trends were examined using chi-square test of independence. For significantly associated pairwise resistance, odds ratios, outlining odd of resistance to one antimicrobial given resistance to another antimicrobial, was calculated using binary logistic regression. Antimicrobials with an overall resistance above 1.5% were plotted in a Circos-based AMR map for each pathogen.

Results
Geographical, temporal, and demographical distribution of cases:

Burden of invasive infections vary between different geographic regions as well as patients’ demographics (1,12).

Hence, we wanted to profile the distribution of bacterial pathogens isolated from blood and CSF cultures over patients’ demographics, geographic location, and morphology of the causative agent. To determine the demographic distribution of invasive infections and the phenotypic characteristics of pathogens, we performed univariate descriptive analyses on 3068 cases as tabulated in Table 1.

Table 1. Attributes of pathogens isolated from blood and cerebrospinal fluid.

| Attribute                                | Number of bacterial pathogens (n = 3068) |
|------------------------------------------|-----------------------------------------|
| Age (years)                               |                                         |
| ≤ 5                                      | 1061 (34.6%)                            |
| 6–18                                     | 262 (8.5%)                              |
| 19–45                                    | 711 (23.2%)                             |
| 46–65                                    | 550 (17.9%)                             |
| > 65                                     | 484 (15.8%)                             |
| Sex                                      |                                         |
| Female                                   | 1245 (40.6%)                            |
| Male                                     | 1823 (59.4%)                            |
| Site of Infection                        |                                         |
| Blood                                    | 2917 (95.1%)                            |
| CSF                                      | 151 (4.9%)                              |
| Year of Isolation                        |                                         |
| 2011                                     | 262 (8.5%)                              |
| 2012                                     | 296 (9.6%)                              |
| 2013                                     | 493 (16.1%)                             |
| 2014                                     | 750 (24.4%)                             |
| 2015                                     | 1267 (41.3%)                            |
| Location of sample collection            |                                         |
| Punjab                                   | 2701 (87.9%)                            |
| KPK                                      | 338 (11%)                               |
| Sindh                                    | 25 (1%)                                 |
| Baluchistan                              | 1 (0%)                                  |
| No Information                           | 3 (1.1%)                                |
| Gram stain                               |                                         |
| Gram-positive bacteria                    | 1433 (46.7%)                            |
| Gram-negative bacteria                    | 1635 (53.3%)                            |

Attributes of pathogens isolated from blood and cerebrospinal fluid (CSF) cultures between 2011 and 2015 in Pakistan. Data are n (%).

A total of 3068 microorganisms were isolated from blood and CSF cultures between 2011 and 2015.

These cases were reported from 44 cities in 4 provinces. Highest number of cases were reported from Punjab (87.9%), followed by Khyber Pakhtunkhwa (11%), Sindh (1%) and Baluchistan (0.033%). The median number of cases from a city was 4 with an interquartile range of 2–15. Out of 44 cities, less than 11 cases were reported from 31 cities during the five-year study period. Highest number of
cases were reported from Lahore (60.5%), Faisalabad (18.3%), and Abbottabad (8.3%). Detailed distribution of cases over geographic location is given in the Supplementary Material (S1 Table).

Temporal and demographical distribution of pathogens isolated from Blood and CSF cultures:
The incidence rates of pathogen-specific invasive infections fluctuate on the basis of their temporal, spatial, and demographical characteristics (13).

Hence, the next logical step was to determine the distribution patterns of pathogens isolated from blood and CSF specimens. To investigate the temporal and demographical distribution of the bacterial species, univariate analyses were performed. Over 75% of these pathogens were from one of the seven most common bacterial species. These included CoNS (41.7%), E. coli (10.6%), Stenotrophomonas. maltophilia (S. maltophilia) (6.2%), Acinetobacter species (6.2%), S. Typhi (6.1%), S. aureus (5.9%), and Klebsiella pneumoniae (K. pneumoniae) (5%). Coagulase-negative Staphylococci was isolated from the highest number of patients throughout the study period. Detailed distribution of common pathogens isolated from blood and CSF cultures is given in Table 2.

Table 2: Profile of common bacterial species isolated from blood and cerebrospinal fluid (CSF) cultures with corresponding demographical and temporal distribution
| Organism                        | < 5 | 6-18 | 19-45 | 46-65 | > 65 | Female | Male | 2011 | 2012 | 2013 | 2014 | 2015 | Total |
|--------------------------------|-----|------|-------|-------|------|--------|------|------|------|------|------|------|-------|
| Coagulase-negative staphylococci | 444 | 74   | 297   | 255   | 209  | 557    | 722  | 101  | 111  | 198  | 337  | 532  | 1279  |
| (41.8%)                        | (28.2%) | (41.8%) | (19.9%) | (43.2%) | (34.1%) | (44.7%) | (39.6%) | (38.5%) | (37.5%) | (40.2%) | (44.9%) | (42%) |
| Escherichia coli               | 57  | 11   | 58    | 90    | 109  | 144    | 181  | 44   | 53   | 73   | 66   | 89   | 325   |
| (5.4%)                         | (4.2%) | (8.2%) | (27.7%) | (22.5%) | (22.9%) | (11.6%) | (9.9%) | (16.8%) | (17.9%) | (14.8%) | (8.8%) | (7%)  | (10.6%) |
| Stenotrophomonas maltophilia   | 215 | ..   | 8     | ..    | 7    | 76     | 160  | ..   | ..   | ..   | 46   | 185  | 236   |
| (20.3%)                        | (1.1%) | (1.1%) | (1.4%) | (1.4%) | (1.4%) | (6.1%) | (8.8%) | (1.4%) | (1.4%) | (1.4%) | (6.1%) | (14.6%) | (7.7%) |
| Acinetobacter species          | 85  | 9    | 41    | 35    | 21   | 81     | 110  | 15   | 16   | 26   | 44   | 90   | 191   |
| (8%)                           | (3.4%) | (5.8%) | (18.3%) | (6.3%) | (18.3%) | (6.5%) | (6%)  | (5.7%) | (5.4%) | (5.3%) | (5.9%) | (7.1%) | (6.2%) |
| Salmonella enterica serovar Typhi | 16 | 82  | 86    | ..    | ..   | 81     | 107  | 13   | 20   | 33   | 58   | 64   | 188   |
| (1.5%)                         | (31.3%) | (12.1%) | (1.4%) | (1.4%) | (1.4%) | (6.5%) | (5.9%) | (5%)  | (6.7%) | (6.7%) | (7.7%) | (5.1%) | (6.1%) |
| Staphylococcus aureus          | 41  | 13   | 52    | 45    | 29   | 71     | 109  | 19   | 20   | 33   | 47   | 61   | 180   |
| (3.9%)                         | (5%)  | (7.3%) | (25%)  | (6%)  | (5.7%) | (6%)  | (6%)  | (7.3%) | (6.8%) | (6.7%) | (6.3%) | (4.8%) | (5.9%) |
| Klebsiella pneumoniae          | 66  | 8    | 26    | 29    | ..   | 53     | 99   | ..   | ..   | 29   | 51   | 71   | 152   |
| (6.2%)                         | (3.1%) | (3.7%) | (19.1%) | (4.8%) | (4.3%) | (5.4%) | (5.4%) | (5.4%) | (5.4%) | (5.4%) | (6.8%) | (5.6%) | (5.6%) |

Data are n (% isolates in a column). Empty cells indicate number of cases < 6. These cases are included in the total.

Temporal and demographical AMR trends in pathogens isolated from blood and CSF cultures

Due to the variations in the treatment approaches as well as the differential ability of pathogens to acquire and disseminate resistance, resistance trends differ in different pathogens. The ability of resistant pathogens to cause infections is also dependent on host-related factors including age, gender, and co-morbidities (14). Hence, after identification of common pathogens, we wanted to determine temporal and demographical AMR trends in them. Susceptibility data was not available for all years throughout the study period on S. maltophilia and K. pneumoniae. Hence, these two pathogens could not be analyzed. Each of the remaining five pathogens was analyzed separately. For each isolate, susceptibility data for all antimicrobials was not available. To account for missing values, available case approach was employed to analyze resistance trends for each antimicrobial. As a result, the number of data points (n) varied between analyses involving different antimicrobials in a pathogen.
In the case of gram-negative organisms, we found out that resistance against fluoroquinolones has increased in E. coli from 50–74.2% between 2011 and 2015 (Table 3 and Fig. 2). Further, an increasing resistance trend against cefipime, a fourth generation cephalosporin, was also observed in E. coli (Table 3 and Fig. 2). While increasing resistance rates were observed for most of the tested antimicrobials, we found decreasing resistance trends against amikacin and gentamicin in E. coli (Table 3 and Fig. 2). Next, increasing resistance trends were observed against most tested antimicrobials in Acinetobacter species (Table 4 and Fig. 3). Of these, the most alarming finding was the steep increase in carbapenem resistance in Acinetobacter species from 50% in 2011 to 95.5% in 2015 (Table 4 and Fig. 3). In the case of S. Typhi, our results have indicated an increasing resistance trend against fluoroquinolones with resistance rates reaching up to 60% in 2015. We have also reported emerging resistance against 3rd and emergence of 4th generation cephalosporins resistance in S. Typhi (Table 5 and Fig. 4). Sex-wise comparisons showed that the rate of isolation of resistant gram-negative pathogens were independent of patients’ sex. Evaluation of age-wise resistance trends highlighted that rate of isolation of resistant pathogens is age dependent. Detailed AMR trends in E. coli, Acinetobacter species, and S. Typhi have been tabulated in Table 3–5, and shown in Figs. 2–4.

Amongst gram-positive pathogens, we found significantly decreasing resistance trends against amikacin, doxycycline, and trimethoprim-sulfamethoxazole in CoNS as well as in S. aureus (Tables 6 and 7; Figs. 5 and 6). While we unexpected did not observe any increasing resistance trend in S. aureus, resistance had increased in CoNS for a range of antimicrobials including 3rd and 4th generation cephalosporins as shown in Table 6 and Fig. 5. Our results also indicated that rate of isolation of resistant gram-positive species varied with patients’ demographic for a wide range of antimicrobials (Tables 6 and 7). Detailed AMR trends in CoNS and S. aureus have been tabulated in Tables 6 and 7, and shown in Figs. 5 and 6, respectively.

Co-resistance trends in pathogens isolated from blood and CSF cultures

Multidrug resistance (MDR) has emerged as a major public health problem globally as well as in Pakistan. Co-resistance to multiple drugs emerges with the selection of strains which are resistant to multiple antimicrobials. Clonal expansion of MDR clones is faster as compared to strains resistant to a
single antimicrobial (15). Hence, it is important to identify those antimicrobials which do not exhibit resistance with any other type of antimicrobials.

To investigate this, antimicrobials belonging to the same class and exhibiting identical resistance profiles in isolates of a given species were merged and entries with missing data were excluded. Chi-square test was used to identify patterns of co-resistance in each pathogen and these patterns are tabulated in Table 8 and Supplementary materials (S2-S6 Tables). Evaluation of E. coli showed that resistance against all antimicrobials was significantly associated except β-lactams, aminoglycosides, fluoroquinolones, and tetracycline. Resistance against these four antimicrobials in E. coli was found to be independent of resistance against other antimicrobials (Table 8 and Fig. 2). We then evaluated Acinetobacter species and our results showed that cefoperazone-sulbactam resistance is not significantly associated with resistance against aminoglycosides, trimethoprim-sulfamethoxazole and tetracycline (Table 8 and Fig. 3). For S. Typhi, we only detected a significant co-resistance between nalidixic acid and fluoroquinolones (Table 8 and Fig. 4). In case of CoNS, we observed a significant co-resistance between all antimicrobials except doxycycline. Doxycycline resistance in CoNS was significantly associated only with pencillin resistance and macrolide resistance (Table 8 and Fig. 5).

Analysis of S. aureus showed that penicillin resistance was not associated with resistance against any other antimicrobials. Furthermore, doxycycline resistance in S. aureus was found to be independent of resistance to all antimicrobials except macrolides and tobramycin (Table 8and Fig. 6). The co-resistance proportions, p-values, and associated odds ratios for all tested antimicrobials are provided in Table 8 and Supplementary materials (S2-S6 Tables). The co-resistance trends are E. coli, Acinetobacter species, S. Typhi, CoNS, and S. aureus have been visualized in Figs. 2–6.

Discussion
Antimicrobial resistance (AMR) has emerged as a major public health concern in both developing and developed countries. Continuous surveillance of AMR has been recommended by World Health Organization (WHO) as a necessary step for controlling emergence of resistance as well as infections caused by resistant pathogens (16). Despite this urgent need to investigate AMR trends, only a handful of studies till date have reported resistance trends in pathogens isolated from blood and CSF
cultures in Pakistan. The current study stands to fill this gap in knowledge of AMR patterns and trends in these bacterial pathogens at a national scale. For that, we undertook a retrospective analysis of AMR of pathogens isolated from clinical specimens of blood and CSF. To the best of our knowledge, this study is the first of its kind in Pakistan providing both demographic and temporal AMR trends in major pathogens from blood and CSF cultures. Our results reflect emergence and rapid increase in rate of isolation of S. maltophilia between 2013 and 2014. Reports from developing as well as the developed countries have also reported a similar trend (17,18). As S. maltophilia is an opportunistic bacteria that infects immunocompromised individuals and hospitalized patients, this trends can be explained by higher survival rates of susceptible patients due to improvements in surgical and healthcare practices as well as extensive usage of broad-spectrum antibiotics. It may also be due to a nosocomial outbreak during the study period (17,19). However, it is difficult to make the statement in the absence of necessary data on comorbidities of our population. Detailed further investigations need to be carried out to assess the risk factors of associated with S. maltophilia infections in Pakistan. Further, as the total number of isolates reported annually fluctuated throughout the study period, the proportion of resistant isolates were determined and analyzed to normalize the data.

Another salient observation of our study is the rapid rise of carbapenems resistance among Acinetobacter species (from 50–95% between 2011 and 2015) in Pakistan. This is in line with earlier CRA trends in Southeast Asian countries (20,21). This increase is concomitant with the two-fold increase in carbapenem usage in Pakistan over the last decade (https://cddep.org/). It is critical to note that carbapenems are one of the few last resort broad-spectrum antibiotics recommended for treatment of sepsis in Pakistan (24) and emergence of CRA has further limited the therapeutic options. Only doxycycline and cefoperazone-sulbactam remain effective against many of the Acinetobacter strains (Table 4). The persistent susceptibility to doxycycline may be attributed to its limited usage in Acinetobacter infections treatment due to its bacteriostatic nature (25). Further, while resistance against cefoperazone-sulbactam was found to be on the rise (Table 4), a wider acquisition of resistance against it might require more time. Additionally, Acinetobacter is an environmental pathogen found in the soil, the acquisition of resistance therefore suggests
inappropriate disposal of antimicrobials. To further explain this, it is likely that unused or expired antimicrobials are being disposed of in a way that they are available to environmental organisms resulting in emergence of resistance in such organism (26,27). This situation provides policymakers with an opportunity to legislate regulated usage of carbapenem, cefoperazone-sulbactam, and doxycycline in the country, and ensure proper disposal of such antimicrobials from hospital systems.

Developed countries like the US and the UK have resorted to using colistin, polymixin B, and tigecycline for the treatment of CRA infections (28,29). Susceptibility data against these antibiotics were not accounted for in this study. Future studies should highlight the susceptibility patterns of Acinetobacter against colistin, polymixin B, and tigecycline.

Table 3
Temporal, age-wise, and gender-wise prevalence of resistant Escherichia coli strains isolated from blood and cerebrospinal fluid (CSF) cultures in Pakistan (2011-2015)

| Antimicrobial | Prevalence (%) of resistant organisms isolated from patients of different age-groups | Prevalence (%) of resistant organisms isolated from males and female patients | Year-wise prevalence (%) of resistant organisms | Total |
|---------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-------|
|                | < 5 Years | 6-18 Years | 19-45 Years | 46-65 Years | > 65 Years | P for difference | Female | Male | P for difference | 2011 | 2012 | 2013 | 2014 | 2015 | P for trend |
| Cefalexin      | 56/5 (100%) | 10/1 (90.9%) | 55/5 (94.8%) | 88/9 (97.8%) | 107/109 (98.2%) | NS | 139/144 (96.5%) | 177/180 (98.3%) | NS | 37/4 (86%) | 52/5 (98.1%) | 72/7 (98.6%) | 66/6 (100%) | 89/8 (100%) | NS | <.0001 |
| Cephradine     | 56/5 (100%) | 10/1 (90.9%) | 55/5 (94.8%) | 88/9 (97.8%) | 107/109 (98.2%) | NS | 139/144 (96.5%) | 177/180 (98.3%) | NS | 37/4 (86%) | 52/5 (98.1%) | 72/7 (98.6%) | 66/6 (100%) | 89/8 (100%) | NS | <.0001 |
| Cefadroxil     | 53/5 (93%) | 10/1 (90.9%) | 54/5 (93.1%) | 82/9 (91.1%) | 107/109 (98.2%) | NS | 133/144 (92.4%) | 168/181 (92.8%) | NS | 40/4 (90.9%) | 52/5 (98.1%) | 68/7 (93.2%) | 56/6 (84.8%) | 85/8 (95.5%) | NS | 316/3 (97.5%) |
| Cefuroxime     | 52/5 (91.2%) | 8/9 (88.9%) | 51/5 (94.4%) | 79/8 (90.8%) | 96/1 (91.4%) | NS | 124/136 (91.2%) | 176/176 (92%) | NS | 28/3 (90.3%) | 49/5 (92.5%) | 68/7 (93.2%) | 56/6 (84.8%) | 85/8 (95.5%) | NS | 286/3 (91.7%) |
| Ampicillin-Sulbactam | 45/5 (90%) | 7/7 (100%) | 35/3 (92.1%) | 61/6 (91%) | 78/8 (88.6%) | NS | 91/1 (90.1%) | 135/144 (90.6%) | NS | 17/2 (81%) | 11/1 (91.7%) | 57/6 (91.9%) | 56/6 (84.8%) | 85/8 (95.5%) | NS | 226/2 (90.4%) |
| Amoxicillin-Clavulanic acid | 51/5 (89.5%) | 10/1 (90.9%) | 54/5 (93.1%) | 82/9 (91.1%) | 95/1 (87.2%) | NS | 129/144 (89.6%) | 163/181 (90.1%) | NS | 33/4 (75%) | 51/5 (96.2%) | 67/7 (91.8%) | 56/6 (84.8%) | 85/8 (95.5%) | NS | 292/3 (89.8%) |
| Ceftriaxone    | 51/5 (89.5%) | 8/11 (72.7%) | 53/5 (91.4%) | 76/9 (84.4%) | 93/1 (95.3%) | NS | 125/144 (86.8%) | 156/181 (86.2%) | NS | 37/4 (84.1%) | 47/5 (88.7%) | 63/6 (86.3%) | 52/6 (78.8%) | 82/8 (92.1%) | NS | 281/3 (96.5%) |
| Drug                  | Cefixime   | Cefoperazone | Trime-Sulfamethoxazole | Cefotaxime | Aztreonam | Cefazidime | Doxycycline | Cefepime | Moxifloxacin | Ofloxacin | Tobramycin | Ciprofloxacin | Levofloxacin | Gentamicin | Amikacin | Piperacillin-Tazobactam | Cefpiperazone-Sulbactam |
|----------------------|------------|--------------|------------------------|------------|-----------|------------|------------|----------|-------------|-----------|------------|---------------|--------------|------------|----------|--------------------------------|------------------------|
| Percentage           | 38/4       | 41/4         | 45/5                   | 51/5       | 45/5      | 49/5       | 36/5       | 44/5     | 27/5        | 29/5      | 45/5       | 28/5          | 29/5         | 40/5      | 19/5    | 30/5                          | 13/5                   |
|                      | (86.4%)    | (87.2%)      | (84.9%)                | (89.5%)    | (86.5%)   | (87.5%)    | (70.6%)    | (77.2%)  | (49.1%)     | (50.9%)   | (80.4%)    | (49.1%)       | (50.9%)      | (70.2%)   | (33.9%) | (18.2%)                         | (22.8%)                |
|                     | 37/4       | 42/4         | 42/4                   | 123/143    | 13/17     | 28/4       | 130/168    | 140/77   | 124/72     | 23/4      | 33/4       | 44/3           | 22/4         | 103/73   | 131/71 | 33/4                          | 13/5                   |
|                      | (88.9%)    | (95.5%)      | (95.5%)                | (86%       | (83.8%)   | (65.1%)    | (84.1%)    | (77.1%)  | (50%)      | (52.3%)   | (67.6%)    | (63.4%)        | (50%)        | (73%)     | (72.4%) | (76.7%)                        | (22.8%)                |
|                     | 8/9        | 82/6        | 82/6                   | 29/4       | 9/15      | 25/8       | 25/18      | 34/6     | 60/10      | 43/5      | 34/5       | 44/3           | 44/1         | 34/7      | 31/10 | 15/1                          | 19/1                   |
|                      | (88.9%)    | (88.6%)     | (88.6%)                | (85.4%)    | (83.2%)   | (62%       | (86.5%)    | (77.3%)  | (62%)      | (62.4%)   | (64.2%)    | (63.4%)        | (60.4%)      | (68.3%)  | (72.4%) | (67.6%)                        | (10.9)          |
|                     | 52/7       | 21/5        | 21/5                   | 123/180    | 7/18      | 44/5       | 180/181    | 180/93   | 172/71     | 46/7      | 57/9       | 32/5           | 32/5         | 80/6     | 57/9   | 57/9                          | 19/1                   |
|                      | (88.9%)    | (81.7%)     | (81.7%)                | (68.3%)    | (62%)     | (68.1%)    | (67.7%)    | (87.2%)  | (71.2%)    | (62.8%)   | (60.8%)    | (60.4%)        | (60.4%)      | (58.6%)  | (57.9) | (57.9)                        | (10.9)          |
|                     | 9/14       | 12/5        | 12/5                   | 29/4       | 9/15      | 25/8       | 25/18      | 34/6     | 60/10      | 43/5      | 34/5       | 44/3           | 44/1         | 34/7      | 31/10 | 15/1                          | 19/1                   |
|                      | (85.7%)    | (95.5%)     | (95.5%)                | (86%       | (83.2%)   | (62%       | (86.5%)    | (77.3%)  | (62%)      | (62.4%)   | (64.2%)    | (63.4%)        | (60.4%)      | (68.3%)  | (72.4%) | (67.6%)                        | (10.9)          |
|                     | 3/8        | 5/5         | 5/5                    | 25/8       | 9/15      | 25/8       | 25/18      | 34/6     | 60/10      | 43/5      | 34/5       | 44/3           | 44/1         | 34/7      | 31/10 | 15/1                          | 19/1                   |
|                      | (37.3%)    | (55.6%)     | (55.6%)                | (62%)      | (62%)     | (62%)      | (68.1%)    | (87.2%)  | (62%)      | (62.4%)   | (64.2%)    | (63.4%)        | (60.4%)      | (68.3%)  | (72.4%) | (67.6%)                        | (10.9)          |
Table 4
Temporal, age-wise and gender-wise prevalence of resistant Acinetobacter species strains isolated from blood and cerebrospinal fluid (CSF) cultures in Pakistan (2011–2015)

| Antimicrobial | Antibiogram (% of resistant group organisms isolated from patients of different age-groups) | Prevalence (% of resistant organisms isolated from males and female patients) | Year-wise prevalence (% of resistant organisms) | Total |
|---------------|-------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|-----------------------------------------------|-------|
|               |                                                                                                 |                                                                                |                                               |       |
| Imipenem      | Carbapenem 4/56 (7.1 %)                                                                          |                                                                                |                                               |       |
|               | 3/11 (27.3 %)                                                                                  |                                                                                |                                               |       |
|               | 3/58 (5.2 %)                                                                                    |                                                                                |                                               |       |
|               | 0/90 (0 %)                                                                                     |                                                                                |                                               |       |
|               | 2/10 (9.1 %)*                                                                                  |                                                                                |                                               |       |
|               | 3/14 (21 %)                                                                                    |                                                                                |                                               |       |
|               | 9/18 (5 %)                                                                                     |                                                                                |                                               |       |
|               | 12/32 (4 %)                                                                                    |                                                                                |                                               |       |
| Meropenem     | Carbapenem 4/56 (7.1 %)                                                                          |                                                                                |                                               |       |
|               | 3/11 (27.3 %)                                                                                  |                                                                                |                                               |       |
|               | 3/58 (5.2 %)                                                                                    |                                                                                |                                               |       |
|               | 0/90 (0 %)                                                                                     |                                                                                |                                               |       |
|               | 2/10 (9.1 %)*                                                                                  |                                                                                |                                               |       |
|               | 3/14 (21 %)                                                                                    |                                                                                |                                               |       |
|               | 9/18 (5 %)                                                                                     |                                                                                |                                               |       |
|               | 12/32 (4 %)                                                                                    |                                                                                |                                               |       |
|               |                                                                                                 |                                                                                |                                               |       |

Data are number of resistant isolates/total number of isolates (%). Denominator varies across cells because all isolates were not tested against the complete set of antimicrobials listed here. Empty cells indicate no cases were tested against the antimicrobial. P-value for difference was calculated by Fisher’s exact test. Bonferroni correction for pairwise comparisons was used to detect differences in percentage of resistant isolates between different age groups. * indicates age groups less susceptible to resistant infections as compared to the age groups labeled with #. P-value for trends was calculated by Cochran Armitage test for trends. Two-sided p-value has been reported. P for trend of < 0.05 indicate an increasing resistance trend unless P-value is followed by “(•)” which shows a decreasing resistance rates against the antimicrobial in Escherichia coli strains. NS: Non significant p-value (i.e. > 0.05).
Antimicrobials

P-value for trends was calculated by Cochran Armitage test for trends. Two-sided p-value has been reported. P for pairwise comparisons was used to detect differences in percentage of resistant isolates between different age groups.

| Tazobactam | Imipenem | Meropenem | Moxifloxacin | Ofloxacin | Trimethoprim-Sulphamethoxazole | Amoxicillin | Ciprofloxacin | Levofloxacin | Tobramycin | Doxycycline | Cefoperazone-Sulbactam |
|------------|----------|-----------|--------------|-----------|-------------------------------|------------|--------------|-------------|------------|-------------|------------------------|
| Inhibitor  | Carbapenem | Carbapenem | Fluoroquinolone | Fluoroquinolone | Sulphadimidine | Aminoglycoside | Fluoroquinolone | Fluoroquinolone | Aminoglycoside | Tetracycline | Cephalosporin Inhibitor |
| (93.6%)    | (90.5%)  | (90.5%)  | (85.7%)      | (84.7%)   | (87.5%)         | (91.4%)    | (88.7%)      | (77.5%)     | (91.4%)    | (65.6%)     | (57.1%)           |
| 7%         | 7%        | 7%        | 7%           | 7%        | 7%               | 7%         | 7%           | 7%          | 7%         | 7%          | 7%               |
| (86.8%)    | (90.2%)  | (90.2%)  | (86.5%)      | (87.5%)   | (87.5%)         | (85.7%)    | (87.5%)      | (87.5%)     | (85.7%)    | (87.5%)     | (87.5%)          |
| (84.4%)    | (91.2%)  | (91.2%)  | (90.5%)      | (90.5%)   | (90.5%)         | (87.5%)    | (87.5%)      | (87.5%)     | (87.5%)    | (87.5%)     | (87.5%)          |
| (85%)      | (80.9%)  | (80.9%)  | (80.9%)      | (80.9%)   | (80.9%)         | (80.9%)    | (80.9%)      | (80.9%)     | (80.9%)    | (80.9%)     | (80.9%)          |
| (90.4%)    | (92.5%)  | (92.5%)  | (92.4%)      | (92.4%)   | (92.4%)         | (92.4%)    | (92.4%)      | (92.4%)     | (92.4%)    | (92.4%)     | (92.4%)          |
| (%)        | (88.2%)  | (88.2%)  | (88.2%)      | (88.2%)   | (88.2%)         | (88.2%)    | (88.2%)      | (88.2%)     | (88.2%)    | (88.2%)     | (88.2%)          |
| (%)        | (75%)     | (75%)     | (75%)        | (75%)     | (75%)            | (75%)      | (75%)        | (75%)       | (75%)      | (75%)       | (75%)             |
| (91.9%)    | (99.9%)  | (99.9%)  | (99.9%)      | (99.9%)   | (99.9%)          | (99.9%)    | (99.9%)      | (99.9%)     | (99.9%)    | (99.9%)     | (99.9%)           |
| (%)        | (97.6%)  | (97.6%)  | (97.6%)      | (97.6%)   | (97.6%)          | (97.6%)    | (97.6%)      | (97.6%)     | (97.6%)    | (97.6%)     | (97.6%)           |
| (%)        | (89.1%)  | (89.1%)  | (89.1%)      | (89.1%)   | (89.1%)          | (89.1%)    | (89.1%)      | (89.1%)     | (89.1%)    | (89.1%)     | (89.1%)           |
| (%)        |          |          |             |           |                  |           |              |             |           |             |                    |

Data are number of resistant isolates/total number of isolates (%). Denominator varies across cells because all isolates were not tested against the complete set of antimicrobials listed here. Empty cells indicate no cases were tested against the antimicrobial. P-value for difference was calculated by Fisher’s exact test. Bonferroni correction for pairwise comparisons was used to detect differences in percentage of resistant isolates between different age groups. * indicates age groups less susceptible to resistant strains as compared to the age-groups labeled with #. P-value for trends was calculated by Cochran Armitage test for trends. Two-sided p-value has been reported. P for trend of < .05 indicates an increasing resistance trend. NS: Non significant p-value (i.e. >.05).

Table 5
Temporal, age-wise and gender-wise prevalence of resistant Salmonella enterica serovar Typhi strains isolated from blood and cerebrospinal fluid (CSF) cultures in Pakistan (2011-2015)

| Antimicrobial group | Prevalence (%) of resistant organisms isolated from patients of different age-groups | Prevalence (%) of resistant organisms isolated from males and female patients | Year-wise prevalence (%) of resistant organisms | Total |
|---------------------|----------------------------------------------------------------------------------|--------------------------------------------------------------------------------|-----------------------------------------------|-------|
| <5                  | P for                                                                            | Femal Male                                                                     | 2011  2012  2013  2014  2015  P for         |       |
| 6-18                | 19-45                                                                            | P for                                                                           |       |       |
| 71/1                | 37.8%                                                                            | <.0001                                                                          |       |       |
| 90/1                | 51.4%                                                                            | NS                                                                               |       |       |
| Antimicrobial | Years | Years | Years | Difference | Difference | Trend | P-value |
|---------------|-------|-------|-------|------------|------------|-------|---------|
| Nalidixic acid | Fluoroquinolone | 12/16 (75%) | 60/65 (92.3%) | 61/64 (95.3%) | 0.03 | 57/63 (90.5%) | 78/85 (91.8%) | NS | 25/26 (96.2%) | 52/58 (89.7%) | 58/64 (90.6%) | NS | 135/148 (91.2%) |
| Moxifloxacin | Fluoroquinolone | 9/14 (64.3%) | 48/77 (62.3%) | 57/82 (69.5%) | NS | 49/77 (63.6%) | 67/100 (67%) | NS | 3/9 (33.3%) | 1/18 (5.6%) | 22/33 (66.7%) | 52/58 (89.7%) | 38/59 (64.4%) | <.001 | 116/117 (65.5%) |
| Ciprofloxacin | Fluoroquinolone | 9/16 (56.3%) | 49/80 (61.3%) | 56/84 (66.7%) | NS | 50/81 (61.7%) | 66/103 (64.1%) | NS | 3/13 (23.1%) | 2/20 (10%) | 22/33 (66.7%) | 52/58 (89.7%) | 37/60 (61.7%) | <.001 | 116/117 (63%) |
| Levofloxacin | Fluoroquinolone | 9/16 (56.3%) | 49/82 (59.8%) | 57/86 (66.3%) | NS | 50/81 (61.7%) | 67/100 (62.6%) | NS | 3/13 (23.1%) | 2/20 (10%) | 22/33 (66.7%) | 52/58 (89.7%) | 38/64 (59.4%) | <.001 | 117/117 (62.2%) |
| Ofloxacin | Fluoroquinolone | 9/16 (56.3%) | 49/82 (59.8%) | 57/86 (66.3%) | NS | 50/81 (61.7%) | 67/100 (62.6%) | NS | 3/13 (23.1%) | 2/20 (10%) | 22/33 (66.7%) | 52/58 (89.7%) | 38/64 (59.4%) | <.001 | 117/117 (62.2%) |
| Trimethoprim-Sulphamides | Sulphamides | 7/16 (43.8%) | 38/77 (49.4%) | 39/80 (48.8%) | NS | 36/76 (47.4%) | 50/104 (49.5%) | NS | 10/13 (76.9%) | 13/20 (65%) | 20/26 (76.9%) | 15/57 (26.3%) | 28/61 (45.9%) | 0.001 | 86/177 (48.6%) |
| Ampicillin | Penicillin | 2/16 (12.5%) | 9/74 (12.2%) | 13/77 (16.9%) | NS | 13/75 (17.3%) | 11/95 (11.6%) | NS | 6/13 (46.2%) | 2/6 (33.3%) | 10/30 (33.3%) | 1/58 (1.7%) | 5/63 (7.9%) | <.001 | 24/17 (14.1%) |
| Ampicillin | Penicillin | 2/16 (12.5%) | 9/74 (12.2%) | 13/77 (16.9%) | NS | 13/75 (17.3%) | 11/95 (11.6%) | NS | 6/13 (46.2%) | 2/6 (33.3%) | 10/30 (33.3%) | 1/58 (1.7%) | 5/63 (7.9%) | <.001 | 24/17 (14.1%) |
| Amoxicillin-Clavulanic acid | Penicillin-Inhibitor | 1/12 (8.3%) | 5/76 (6.6%) | 11/79 (13.9%) | NS | 10/72 (13.9%) | 7/98 (7.1%) | NS | 3/13 (23.1%) | 5/20 (25%) | 6/33 (18.2%) | 0/50 (0%) | 3/54 (5.6%) | 0.000 | 17/17 (10%) |
| Ampicillin-Sulbac tam | Penicillin-Inhibitor | 1/11 (9.1%) | 4/60 (6.7%) | 5/54 (9.3%) | NS | 6/53 (11.3%) | 4/74 (5.4%) | NS | 2/3 (66.7%) | 1/6 (16.7%) | 4/14 (28.6%) | 0/50 (0%) | 3/54 (5.6%) | 0.000 | 10/12 (7.9%) |
| Ceftriaxone | Cephalosporins | 0/16 (0%) | 1/81 (1.2%) | 4/84 (4.8%) | NS | 4/81 (4.9%) | 1/104 (1%) | NS | 1/13 (7.7%) | 1/20 (5%) | 1/33 (3%) | 0/56 (0%) | 2/63 (3.2%) | 5/185 (2.7%) |
| Cefotaxime | Cephalosporins | 0/16 (0%) | 1/81 (1.2%) | 4/85 (4.7%) | NS | 4/80 (5%) | 1/106 (0.9%) | NS | 1/13 (7.7%) | 1/20 (5%) | 1/32 (3.1%) | 0/57 (0%) | 2/64 (3.1%) | 5/186 (2.7%) |
| Cefixime | Cephalosporins | 0/16 (0%) | 0/69 (0%) | 3/70 (4.3%) | NS | 3/67 (4.5%) | 0/91 (0%) | NS | 0/6 (0%) | 1/32 (3.1%) | 0/56 (0%) | 2/64 (3.1%) | 3/158 (1.9%) |
| Cefoperazone | Cephalosporins | 0/16 (0%) | 0/74 (0%) | 3/74 (4.1%) | NS | 3/69 (4.3%) | 0/98 (0%) | NS | 0/13 (0%) | 1/32 (3.1%) | 0/56 (0%) | 2/64 (3.1%) | 3/167 (1.8%) |
| Ceftazidime | Cephalosporins | 0/16 (0%) | 0/79 (0%) | 3/85 (3.5%) | NS | 3/81 (3.7%) | 0/103 (0%) | NS | 0/13 (0%) | 0/20 (0%) | 1/33 (3%) | 0/56 (0%) | 2/62 (3.2%) | NS | 3/184 (1.6%) |
| Cefepime | Cephalosporins | 0/7 (0%) | 0/53 (0%) | 1/40 (2.5%) | NS | 1/44 (2.3%) | 0/58 (0%) | NS | 0/11 (0%) | 0/20 (0%) | 0/4 (0%) | 0/17 (0%) | 1/50 (2%) | NS | 1/102 (1%) |

Data are number of resistant isolates/total number of isolates (%). Less than 5 isolates were isolated from patients above 45 year of age and are not shown in the table. Denominator varies across cells because all isolates were not tested against the complete set of antimicrobials listed here. Empty cells indicate no cases were tested against the antimicrobial. P-value for difference was calculated by Fisher’s exact test. Bonferroni correction for pairwise comparisons was used to detect differences in percentage of resistant isolates between different age groups. * indicates age groups less susceptible to resistant infections as compared to the age groups labeled with #. P-value for trends was calculated by Cochran Armitage test for trends. Two-sided p-value has been reported. P for trend of < 0.05 indicate an increasing resistance trend unless P-value is followed by “(-)” which shows a decreasing resistance rates against the antimicrobial in Salmonella enterica serovar Typhi strains. NS: Non-significant p-value.
resistance rates against the antimicrobial in Salmonella enterica Serovar Typhi strains. NS: Non-significant p-value (i.e., >0.05).

Table 6. Temporal, age-wise and gender-wise prevalence of resistant Coagulase-negative Staphylococci strains isolated from blood and cerebrospinal fluid (CSF) cultures in Pakistan (2011-2015)

| Antimicrobials | Antimicrobial group | Prevalence (%) of resistant organisms isolated from patients of different age-groups | Prevalence (%) of resistant organisms isolated from males and female patients |Year-wise prevalence (%) of resistant organisms | Total |
|----------------|---------------------|-----------------------------------------------------------------------------------|----------------------------------------------------------------------------------|-----------------------------------------------|-------|
|                | <5 Years | 6-18 Years | 19-45 Years | >65 Years | P for difference | Female | Male | P for difference | 2011 | 2012 | 2013 | 2014 | 2015 | P for trend |
| Amoxicillin    | Penicillin          | 370/441 (83.9%) # | 527/649 (83.1%) # | 271/309 (90.1%) # | 191/254 (75.2%) # | 154/207 (74.4%) # | <.001 | 409/556 (73.6%) | 568/717 (79.2%) | 84/101 (83.2%) | 88/109 (80.7%) | 179/217 (90.9%) | 244/336 (72.6%) | 382/530 (72.1%) | <.001 | 977/1273 (76.7%) |
| Ampicillin     | Penicillin          | 367/438 (83.8%) # | 527/649 (83.1%) # | 271/309 (90.1%) # | 191/254 (75.2%) # | 153/206 (73.4%) # | <.001 | 407/554 (73.5%) | 564/713 (79.1%) | 83/100 (83%) | 87/108 (80.6%) | 176/214 (90.7%) | 244/336 (72.6%) | 381/529 (72%) | <.001 | 971/1267 (76.6%) |
| Azithromycin   | Macrolide           | 359/437 (82.2%) # | 447/547 (82.1%) # | 271/309 (90.1%) # | 191/254 (75.2%) # | 160/204 (78.4%) # | <.001 | 422/552 (76.4%) | 542/707 (76.7%) | 74/94 (78.7%) | 78/110 (70.9%) | 154/198 (77.8%) | 257/334 (76.9%) | 401/523 (76.7%) | NS | 964/1259 (76.6%) |
| Erythromycin   | Macrolide           | 356/434 (82.0%) # | 447/547 (82.1%) # | 271/309 (90.1%) # | 191/254 (75.2%) # | 159/203 (78.3%) # | <.001 | 420/559 (76.5%) | 539/704 (76.6%) | 74/94 (78.7%) | 78/110 (70.9%) | 153/197 (77.7%) | 256/333 (76.9%) | 398/519 (76.7%) | NS | 959/1253 (76.5%) |
| Trimethoprim    | Sulphonamides       | 286/343 (66.0%) # | 407/547 (54.3%) # | 202/245 (66.1%) # | 157/245 (63.1%) # | 136/201 (67.7%) # | NS | 362/547 (66.2%) | 459/695 (66%) | 92/100 (92%) | 82/107 (74.5%) | 137/185 (74.1%) | 206/337 (61.1%) | 304/510 (59.6%) | <.001 | 821/1242 (66.1%) |
| Sulphamethoxazole | Sulphonamides       | 286/343 (66.0%) # | 407/547 (54.3%) # | 202/245 (66.1%) # | 157/245 (63.1%) # | 136/201 (67.7%) # | NS | 362/547 (66.2%) | 459/695 (66%) | 92/100 (92%) | 82/107 (74.5%) | 137/185 (74.1%) | 206/337 (61.1%) | 304/510 (59.6%) | <.001 | 821/1242 (66.1%) |
| Cephradine      | Cephalosporin       | 306/443 (69.1%) # | 297/397 (75.7%) # | 157/297 (52.9%) | 148/255 (58%) | 114/209 (54.5%) | <.001 | 318/557 (57.1%) | 436/720 (60.6%) | 83/101 (82.2%) | 67/110 (60.9%) | 107/197 (51.3%) | 192/337 (57%) | 311/532 (58.5%) | 0.001 | 754/1277 (59%) |
| Cephalalexin    | Cephalosporin       | 307/444 (69.1%) # | 297/397 (75.7%) # | 157/297 (52.9%) | 148/255 (58%) | 114/209 (54.5%) | <.001 | 318/557 (57.1%) | 437/722 (60.5%) | 83/101 (82.2%) | 67/110 (60.4%) | 108/197 (51.5%) | 192/337 (57%) | 311/532 (58.5%) | 0.001 | 755/1279 (59%) |
| Cefaclor        | Cephalosporin       | 306/443 (69.1%) # | 297/397 (75.7%) # | 157/297 (52.9%) | 148/255 (58%) | 113/208 (54.3%) | <.001 | 315/556 (56.7%) | 437/719 (60.1%) | 83/101 (82.2%) | 63/107 (57.3%) | 99/197 (50.3%) | 191/335 (57%) | 311/532 (58.5%) | 0.001 | 747/1275 (59.6%) |
| Cefazidime      | Cephalosporin       | 305/444 (68.7%) # | 297/397 (75.7%) # | 157/297 (52.9%) | 148/255 (58%) | 112/208 (53.8%) | <.001 | 310/556 (55.8%) | 430/722 (59.6%) | 83/101 (82.2%) | 56/111 (50.5%) | 98/198 (49.5%) | 192/336 (57.1%) | 311/532 (58.5%) | NS | 740/1278 (57.9%) |
| Ceftriaxone     | Cephalosporin       | 304/443 (68.6%) # | 297/397 (75.7%) # | 157/297 (52.9%) | 148/255 (58%) | 112/209 (53.6%) | <.001 | 309/556 (55.6%) | 429/721 (59.5%) | 83/101 (82.2%) | 56/111 (50.5%) | 97/197 (49.2%) | 192/336 (56.8%) | 311/532 (58.5%) | NS | 738/1277 (57.8%) |
| Amoxicillin     | Penicillin          | 301/297 (104) # | 153/143 (104) # | 153/143 (104) # | 112/209 (104) # | 309/297 (104) # | NS | 83/155 (54.3%) | 97/157 (59.8%) | 97/160 (59.4%) | 97/207 (47.5%) | 192/311 (60.1%) | NS | 738/1278 (57.9%) |
| Inhibitor          | Inhibitor          | Inhibitor          | Inhibitor          | Inhibitor          | Inhibitor          |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Tobramycin        | Gentamicin        | Meropenem         | Imipenem          | Fusid acid        | Aminoglycoside    |
| Aminoglycoside    | Aminoglycoside    | Aminoglycoside    | Aminoglycoside    | Fusid acid        | Aminoglycoside    |
|                   |                   |                   |                   |                   |                   |
| 233/438           | 233/438           | 225/444           | 225/444           | 198/419           | 233/438           |
| 13/7              | 13/7              | 13/7              | 13/7              | 13/7              | 13/7              |
| 962/96           | 962/96           | 962/96           | 962/96           | 962/96           | 962/96           |
| 90/2              | 90/2              | 90/2              | 90/2              | 90/2              | 90/2              |
| 54                | 54                | 54                | 54                | 54                | 54                |
| 0.001             | 0.001             | 0.001             | 0.001             | 0.001             | 0.001             |
| 0.001             | 0.001             | 0.001             | 0.001             | 0.001             | 0.001             |
| In-Clavulanic acid| In-Clavulanic acid| In-Clavulanic acid| In-Clavulanic acid| In-Clavulanic acid| In-Clavulanic acid|
| Ampicilli         | Ampicilli         | Ampicilli         | Ampicilli         | Ampicilli         | Ampicilli         |
| Meropenem         | Meropenem         | Meropenem         | Meropenem         | Meropenem         | Meropenem         |
| 25/6              | 25/6              | 25/6              | 25/6              | 25/6              | 25/6              |
| 34/1              | 34/1              | 34/1              | 34/1              | 34/1              | 34/1              |
| 136/96            | 136/96            | 136/96            | 136/96            | 136/96            | 136/96            |
| 0.005             | 0.005             | 0.005             | 0.005             | 0.005             | 0.005             |
| 0.005             | 0.005             | 0.005             | 0.005             | 0.005             | 0.005             |
| Ciproflaxacin     | Ciproflaxacin     | Ciproflaxacin     | Ciproflaxacin     | Ciproflaxacin     | Ciproflaxacin     |
| Fluoroquinolone   | Fluoroquinolone   | Fluoroquinolone   | Fluoroquinolone   | Fluoroquinolone   | Fluoroquinolone   |
| 282/444           | 282/444           | 282/444           | 282/444           | 282/444           | 282/444           |
| 3/3               | 3/3               | 3/3               | 3/3               | 3/3               | 3/3               |
| 14/27             | 14/27             | 14/27             | 14/27             | 14/27             | 14/27             |
| 257/555           | 257/555           | 257/555           | 257/555           | 257/555           | 257/555           |
| 0.001             | 0.001             | 0.001             | 0.001             | 0.001             | 0.001             |
| 0.001             | 0.001             | 0.001             | 0.001             | 0.001             | 0.001             |
| Levofloxacin      | Levofloxacin      | Levofloxacin      | Levofloxacin      | Levofloxacin      | Levofloxacin      |
| Fluoroquinolone   | Fluoroquinolone   | Fluoroquinolone   | Fluoroquinolone   | Fluoroquinolone   | Fluoroquinolone   |
| 271/426           | 271/426           | 271/426           | 271/426           | 271/426           | 271/426           |
| 9/9               | 9/9               | 9/9               | 9/9               | 9/9               | 9/9               |
| 147/280           | 147/280           | 147/280           | 147/280           | 147/280           | 147/280           |
| 0.001             | 0.001             | 0.001             | 0.001             | 0.001             | 0.001             |
| 0.001             | 0.001             | 0.001             | 0.001             | 0.001             | 0.001             |
| Cefixime          | Cefixime          | Cefixime          | Cefixime          | Cefixime          | Cefixime          |
| Cephalosporin     | Cephalosporin     | Cephalosporin     | Cephalosporin     | Cephalosporin     | Cephalosporin     |
| 270/396           | 270/396           | 270/396           | 270/396           | 270/396           | 270/396           |
| 6%                | 6%                | 6%                | 6%                | 6%                | 6%                |
| 197/415           | 197/415           | 197/415           | 197/415           | 197/415           | 197/415           |
| 0.001             | 0.001             | 0.001             | 0.001             | 0.001             | 0.001             |
| 0.001             | 0.001             | 0.001             | 0.001             | 0.001             | 0.001             |
| Cefuroxime        | Cefuroxime        | Cefuroxime        | Cefuroxime        | Cefuroxime        | Cefuroxime        |
| Cephalosporin     | Cephalosporin     | Cephalosporin     | Cephalosporin     | Cephalosporin     | Cephalosporin     |
| 287/422           | 287/422           | 287/422           | 287/422           | 287/422           | 287/422           |
| 6%                | 6%                | 6%                | 6%                | 6%                | 6%                |
| 187/411           | 187/411           | 187/411           | 187/411           | 187/411           | 187/411           |
| 0.001             | 0.001             | 0.001             | 0.001             | 0.001             | 0.001             |
| 0.001             | 0.001             | 0.001             | 0.001             | 0.001             | 0.001             |
Data are number of resistant isolates/total number of isolates (percentage of resistant isolates).

Denominator varies across cells because all isolates were not tested against the complete set of antimicrobials listed here. Empty cells indicate no cases were tested against the antimicrobial. P-value for difference was calculated by Fisher’s exact test. Bonferroni correction for pairwise comparisons was used to detect differences in percentage of resistant isolates between different age groups. * indicates age-groups less susceptible to resistant strains as compared to the age-groups labeled with #. P-value for trends was calculated by Cochran Armitage test for trends. Two-sided p-value has been reported. P for trend of <0.05 indicate an increasing resistance trend unless P-value is followed by “(-)” which shows a decreasing resistance rates against the antimicrobial. NS: Non significant p-value (i.e. >0.05)

Table 7
Temporal, age-wise and gender-wise prevalence of resistant Staphylococcus aureus strains isolated from blood and cerebrospinal fluid (CSF) cultures in Pakistan (2011–2015).

| Antibiotic     | Prevalence (%) of resistant organisms isolated from patients of different age-groups | Prevalence (%) of resistant organisms isolated from males and female patients | Year-wise prevalence (%) of resistant organisms | Total |
|----------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|-----------------------------------------------|-------|
|                | <5  | 6-18Y | 19-45Y | 46-65Y | >65Y | P for difference | Male | P for difference | 2011 | 2012 | 2013 | 2014 | 2015 | P for Trend |
| Ampicillin     | 40/4 | 12/1  | 12/1   | 20/2   | NS            | 64/7 | 105/9 | NS | 16/1 | 17/2 | 33/3 | 46/4 | 57/6 | NS | 169/1 | 80 (93.9 %) |
| Penicillin     | 40/4 | 12/1  | 12/1   | 20/2   | NS            | 64/7 | 105/9 | NS | 16/1 | 17/2 | 33/3 | 46/4 | 57/6 | NS | 169/1 | 80 (93.9 %) |
| Tobra          | 19/3 | 18/2  | 22/3   | 35/5   | NS            | 10/1 | 5/7    | 11/1 | 29/4 | 30/5 | NS | 85/14 | 18 |

Table 7 (continued):

| Antimicrobial | Denominator | P for Trend |
|---------------|-------------|-------------|
| Amikacin      | Denominator | P for Trend |
| Tobra         | Denominator | P for Trend |

| Clindamycin  | Lincosamide | Doxycycline | Amikacin    |
|-------------|-------------|-------------|-------------|
| Denominator | P for Trend |
| Amikacin    | Tobra       | Amikacin    | Tobra       |

P for trend of <0.05 indicate an increasing resistance trend unless P-value is followed by “(-)” which shows a decreasing resistance rates against the antimicrobial. NS: Non significant p-value (i.e. >0.05)
| Drug                          | Ne + erazo | Sulph mylene | Cefop | Ampi | Ceftri | zidim | Cefacl | m | Mero |
|-------------------------------|------------|--------------|-------|------|-------|-------|-------|---|------|
| Trime thoprim + Sulpha mest | 29/3       | 6/12         | 31/5  | 20/4 | 15/2  | NS    | 31/6  | 5 | 6/1  |
| Erythromycin Macrolides      | 22/3       | 6/13         | 22/5  | 21/4 | 17/2  | NS    | 29/6  | 9 | 1/1  |
| Azithromycin Macrolides      | 23/3       | 6/13         | 22/5  | 20/4 | 14/2  | NS    | 28/6  | 9 | 0/1  |
| Levof loxacin Fluorquinolone | 12/3       | 3/12         | 25/4  | 23/4 | 16/2  | NS    | 26/6  | 8 | 1/1  |
| Moxifloxacin Fluorquinolone  | 12/3       | 4/12         | 21/4  | 21/3 | 15/2  | NS    | 24/6  | 4 | 1/1  |
| Amoxicillin-Clavulanic acid  | 20/4       | 4/13         | 24/5  | 19/4 | 16/2  | NS    | 29/7  | 1 | 4/1  |
| Ofloxacin Fluorquinolone     | 13/4       | 3/13         | 25/5  | 24/4 | 17/2  | NS    | 27/7  | 1 | 5/1  |
| Imip em Carbenem             | 20/4       | 4/13         | 24/5  | 19/4 | 15/2  | NS    | 29/7  | 1 | 4/1  |
| Mero penem Carbenem          | 20/4       | 4/13         | 24/5  | 19/4 | 15/2  | NS    | 29/7  | 1 | 4/1  |
| Cefot or Cephalorins         | 20/4       | 4/13         | 24/5  | 19/4 | 15/2  | NS    | 29/7  | 1 | 4/1  |
| Cefta zidine Cephalorins     | 20/4       | 4/13         | 24/5  | 19/4 | 15/2  | NS    | 29/7  | 1 | 4/1  |
| Ceftri axone Cephalorins     | 20/4       | 4/13         | 24/5  | 19/4 | 15/2  | NS    | 29/7  | 1 | 4/1  |
| Ceph alexin Cephalorins      | 20/4       | 4/13         | 24/5  | 19/4 | 15/2  | NS    | 29/7  | 1 | 4/1  |
| Cip rofloxacin Fluorquinolone| 4/14       | 3/13         | 25/5  | 23/4 | 17/2  | NS    | 26/7  | 1 | 5/1  |
| Ampicillin-Sulbactam         | 20/3       | 4/13         | 16/4  | 14/2 | 9/20  | NS    | 24/6  | 2 | 4/1  |
| Cefep im Cephalorins         | 18/3       | 4/12         | 17/4  | 15/3 | 7/20  | NS    | 20/6  | 0 | 4/1  |
| Cefop erazone Cephalorins    | 18/3       | 4/12         | 17/4  | 15/3 | 7/20  | NS    | 20/6  | 0 | 4/1  |
### Table 8

Selected co-resistance trends in pathogens isolated from blood and cerebrospinal fluid (CSF) cultures in Pakistan (2011-2015).

| Pathogen         | Antimicrobial 1 | Antimicrobial 2 | Number of isolates resistant to antimicrobial 1 (%) | Number of isolates resistant to antimicrobial 1 & 2 (%) | Number of isolates resistant to antimicrobial 2 (%) | P for difference | Odds ratio (95% CI) | Antimicrobial -lactams                |
|------------------|-----------------|-----------------|-----------------------------------------------------|--------------------------------------------------------|-----------------------------------------------------|------------------|----------------------|---------------------------------------|
| Coagulase-       | Doxycycline     |                 |                                                     |                                                        |                                                     |                  |                      | Amikacin                                     |
| negative         |                 |                 |                                                     |                                                        |                                                     |                  |                      | β-lactams                                         |
| Staphylococci    |                 |                 |                                                     |                                                        |                                                     |                  |                      | Clindamycin                                   |
|                  | 13/198 (6.6%)   | 13/64 (1.4%)    | 0.5091                                              | 0.81 (0.43-1.52)                                       |                                                    |                  |                      | Fusidic acid                                  |
|                  | 12/198 (62.1%)  | 12/474 (25.9)   | 0.0779                                              | 1.34 (0.97-1.86)                                       |                                                    |                  |                      | Gentamicin                                     |
|                  | 17/20 (85.0%)   | 17/20 (85.0%)   |                                                     |                                                        |                                                    |                  |                      | Tobramycin                                     |
| Acinetobacter    | Cephalosporins  |                 |                                                     |                                                        |                                                     |                  |                      | Piperacillin-tazobactam                      |
|                  | 54/54 (100%)    | 54/104 (1.9%)   | 0.118                                               | NA                                                     |                                                    |                  |                      | Amikacin                                     |
| Antibiotic/Resistance Class | Organism | % Susceptibility | MIC (μg/mL) | Frequency of Resistance | MIC Range (μg/mL) |
|-----------------------------|----------|-----------------|------------|------------------------|------------------|
| Tobramycin                  | 79/106 (74.5) | 2/5 (40) | 0.0703 | Tobramycin | 9 (0.9–90.33) |
| Gentamicin                  | 99/106 (99) | 1/1 (100) | 0.1433 | Amikacin | 14.14 (0.8–250.9) |
| Tramethoprim-sulphamethoxazole | 79/106 (93.4) | 0/1 (0) | 0.073 | Tobramycin | 34.33 (1.71–689.68) |
| Cephalosporin               | 79/106 (75) | 0/1 (0) | 0.0583 | Cephalosporin | 3.33 (0.9–12.28) |
| Carbapenem                  | 79/106 (74.5) | 2/5 (40) | 0.0703 | Tobramycin | 9 (0.9–90.33) |
| Piperacillin-tazobactam     | 79/106 (75) | 0/1 (0) | 0.0583 | Tobramycin | 3.33 (0.9–12.28) |
| Doxycycline                 | 51/55 (92.7) | 0/1 (0) | 0.196 | Cefoperazone-sulbactam | 2.27 (0.64–8.03) |
| Tobramycin                  | 72/79(91.1) | 0/1 (0) | 0.2984 | Trimethoprim-sulphamethoxazole | 2.14 (0.62–7.38) |
| Escherichia coli Carbapenem | 3/6 (50) | 0/1 (0) | 0.1118 | Doxycycline | 0.25 (0.05–1.32) |
| 6/6 (100) | 0.181 | Tobramycin | 0.25 (0.05–1.32) |
| 5/6 (83.3) | 0.2367 | Gentamicin | 3.96 (0.45–34.62) |
| 6/6 (100) | 0.3604 | 4th generation cephalosporin | 0.45–34.62 |
| 6/6 (100) | 0.3732 | NA | 0.45–34.62 |
| 6/6 (100) | 0.5935 | NA | Trimethoprim-sulphamethoxazole | 0.45–34.62 |
| 6/6 (100) | 0.5958 | NA | 0.45–34.62 |
| 6/6 (100) | 0.5958 | NA | 0.45–34.62 |
| 6/6 (100) | 0.6462 | NA | 0.45–34.62 |
| 6/6 (100) | 0.6462 | NA | 0.45–34.62 |
| 6/6 (100) | 0.6572 | NA | 0.45–34.62 |
| 6/6 (100) | 1.63 | NA | 0.45–34.62 |
| 5/6 (83.3) | 0.181 | Tobramycin | 0.25 (0.05–1.32) |
| 12/15 (80) | 0.0575 | Gentamicin | 3.33 (0.9–12.28) |
| 15/15 (100) | 0.0772 | 4th generation cephalosporin | 0.18–14.33 |
| 15/15 (100) | 0.1308 | NA | 0.18–14.33 |
| Cefoperazone-sulbactam      | 12/15 (80) | 0.0575 | Gentamicin | 3.33 (0.9–12.28) |
| 15/15 (100) | 0.0772 | 4th generation cephalosporin | 0.18–14.33 |
| 15/15 (100) | 0.1308 | NA | 0.18–14.33 |
| Antibiotic Combination                      | Frequency | Minimal Inhibitory Concentration (MIC) | Description |
|--------------------------------------------|-----------|----------------------------------------|-------------|
| Piperacillin-tazobactam                    | 17/17(100)| 0.0795 NA                               | 3rd generation cephalosporin |
| Amikacin                                   | 15/22(68.2)| 0.2613 0.53 (0.2-1.41)                  | Doxycycline |
| Gentamicin                                 | 78/96(81.3)| 0.3528 1.42 (0.68-2.97)                 | Doxycycline |
| Salmonella enterica serovar Typhi          | 4/5(80)   | 0.172 5.24 (0.567-48.55)                | Trimethoprim-sulphamethoxazole |
| Penicillin                                 | 4/5(80)   | 0.356 0.33 (0.053-3.348)                | Nalidixic acid |
| Trimethoprim-sulphamethoxazole             | 46/49(93.8)| 0.51 1.704 (0.403-7.195)               | Nalidixic acid |
| Staphylococcus aureus                      | 7/24(29.2) | 0.0502 2.97 (0.97-9.14)                 | Amikacin    |
| Doxycycline                                | 15/24(62.5)| 0.1273 2.07 (0.8-5.33)                 | Macrolides  |
| Antimicrobial | Resistant to Both Antimicrobials | Resistant to Antimicrobial 1 | Resistant to Antimicrobial 2 | P-value | Odds-Ratio | 95% CI |
|---------------|---------------------------------|-----------------------------|-----------------------------|---------|------------|--------|
| 8-lactam (except penicillin) | 13/24 (54.2) | 13/44 (29.5) | 0.2935 | 1.64 | (0.65–4.14) |
| Gentamicin    | 11/24 (45.8) | 11/37 (29.7) | 0.3475 | 1.56 | (0.61–3.98) |
| Fluoroquinolone | 12/24 (50) | 12/47 (25.5) | 0.8179 | 1.11 | (0.44–2.8) |
| Trimethoprim-sulphamethoxazole | 25/44 (56.8) | 25/48 (52.1) | 0.1612 | 1.77 | (0.79–3.96) |
| Macrolides    | 8/44 (18.2) | 8/16 (50) | 0.6538 | 1.28 | (0.44–3.74) |
| Amikacin      | 58/94 (61.1) | 58/50 (98.3) | 0.5613 | 3.14 | (0.27–35.81) |

Data are number of isolates resistant to both antimicrobials / number of isolates resistant to either antimicrobial 1 (in the case of R1) or antimicrobial 2 (in the case of R1) (%). P-value for difference was calculated using Chi-square test. Odds-ratio was calculated using binary logistic regression and is listed with 95% confidence interval (95% CI). Two-sided p-value has been reported.

Another important finding of this study is the emergence of third and fourth generation cephalosporins resistance in S. Typhi. Recent studies have reported an incidence of third generation resistant S. Typhi infections in Pakistan and neighboring countries (30,31). These antimicrobials are the drugs of choice for the empirical treatment of these infections (32). This suggests that treatment of these infections with cephalosporins will become ineffective. Case studies from Canada and the US have shown that these infections can successfully be treated with carbapenems and ceftriaxone-azithromycin combination (33). However, it is highly likely that S. Typhi will acquire resistance against these antimicrobials in the short term. In the light of this, WHO has recommended large scale implementation of Typbar-TCV, a typhoid vaccine, for the containment of S. Typhi infections in high risk regions (32,34). Improved personal hygiene, handwashing, availability of clean drinking water, and well cooked food lowers the risk of S. Typhi infections by 20% (32,35). The government should actively work towards implementing these suggestions to limit the spread of drug resistant S. Typhi infections.

While AMR was rising in other major pathogens, we unexpectedly found decreasing resistance rates against few major antimicrobials in S. aureus. This is in line with the reports on incidence of antimicrobial resistant S. aureus infections worldwide (36,37). While the exact reason for this decrease is yet to be identified, studies have suggested that it may be due to improved infection control practices and improved antibiotic prescription guidelines (36). The ability of S. aureus to excise resistance markers, out of its genome, in the absence of antimicrobials may have played a role in the decreasing resistance as well. Excision of resistance markers is associated with reduced
metabolic cost and overall increase in the bacterial fitness (36,38,39). While the current resistance reported levels in S. aureus are low, continuous surveillance is required to keep track of emerging resistance trends in this pathogen.

This study is limited in that two provinces, namely Baluchistan and Gilgit-Baltistan, were not represented. However, as the combined population of these two provinces constitutes less than 10% of the population of Pakistan, our study is representative of over 90% of the population (40). Next, while our results have shown CoNS to be the most prevalent bacterial pathogen isolated from the blood and CSF cultures, this can be explained by CoNS being a common skin contaminant. However, further studies needs to be carried to determine the role of CoNS as a potential bacteremia pathogen. In addition, studies should also focus on limiting the contamination of clinical samples with CoNS to prevent false diagnosis. Lastly, susceptibility data on last resort antibiotics, including colistin, polymixin B, and linezolid, was not available. Susceptibility patterns of invasive pathogens to these antibiotics still need to be carried out.

Conclusions
In this study we set out to determine resistance patterns in pathogens isolated from blood and CSF cultures. We found that resistance has been at rise for several of these pathogens. Highest resistance rates were observed in Acinetobacter species against all tested antimicrobials including carbapenems. Resistance against 3rd and 4th generation cephalosporins has been reported in S. Typhi during the study period. Policy makers should prioritize and expedite implementation of infection control practices and antimicrobial stewardship in the country to control the emerging threat of AMR to public health.

Abbreviations
AMR
Antimicrobial resistance
CSF
Cerebrospinal fluid
CL
Chughtai Laboratory
API
Analytical Profile Index
CoNS
Coagulase-negative staphylococci

Declarations

Ethics approval

As this study involved secondary data analysis of de-identified patient isolates, it was exempted from the Institutional Review Board (IRB). However, a Memorandum of Understanding (MoU) was signed between Chughtai Lab and Lahore University of Management Sciences.

Consent for publication

Not applicable

Availability of data and materials

Complete dataset analyzed in the current study is included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

SM conceptualized, designed, and supervised the study. QS and KR performed the microbiological experiments for species identification and determining their resistance patterns. NJ, SUC, and FA performed the formal analysis while NJ and SUC worked on data visualization. SG provided guidance
on study design, data analyses, and results interpretation. NJ, SUC, and SM wrote the manuscript. All authors contributed to the interpretation of results, editing of the manuscript and approved the final version.

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Additional Files

Additional file 1 .doc. Distribution of bacterial isolates from blood and cerebrospinal fluid (CSF) cultures from different cities.
Additional file 2.doc. Co-resistance patterns in *Escherichia coli*.

Additional file 3.doc. Co-resistance patterns in *Acinetobacter*.

Additional file 4.doc. Co-resistance patterns in *Salmonella enterica* serovar Typhi.

Additional file 5.doc. Co-resistance patterns in Coagulase-negative Staphylococci.

Additional file 6.doc. Co-resistance patterns in *Staphylococcus aureus*.

Additional file 7.xlsx. Complete dataset

Figures
Susceptibility records obtained from CLL  
N = 3092

Exclusion of 5 records on pathogens from sites other than blood and cerebrospinal fluid (CSF)  
N = 3087

Exclusion of 19 records with missing demographics  
N = 3068

Descriptive univariate analysis of the cases  
N = 3068

Proportion of the bacterial species identified  
N = 3068

Exclusion of records on pathogens with relative frequency ≤3%  
N=2551

Agewise, genderwise and yearwise distribution of pathogens  
N=2551

Exclusion of records on pathogens not isolated every year  
N = 2163

Pathogen-wise analysis on:
1. CoNS  (n=1279)  
2. E. coli  (n=325)  
3. Acinetobacter spp  (n=191)  
4. S. Typhi  (n=188)  
5. S. aureus  (n = 180)
Figure 1
Flow of cases. Flow of the cases through the study has been shown.

Figure 2
Antimicrobial resistance in Escherichia coli (E. coli) A: Each section of the diagram represents the resistance observed in E. coli against the antibiotic. Size of each section is proportional to the proportion of E. coli resistant to the antibiotic over the study period. Antibiotics of the same class are shown in similar colors. B: Line graphs show temporal
trends of proportion of resistant E. coli in a clockwise direction from 2011 to 2015. C: Bar charts show the comparison of susceptibility to resistant strains in patients of different age groups. Moving from out to inward, bars represent proportion of resistant E. coli reported in children <5 years of age, young adults between 6 to 18 years, middle aged 19 to 45 years old, 45 to 65 years old patients, and elderly over 65 years of age, respectively. D: Gender-wise comparison to susceptibility to resistant E. coli is shown in form bars. Outer circle and inner circle shows proportion of resistant E. coli isolated from women vs. men, respectively.

E: For co-resistance analysis, antibiotics belonging to the same class with same susceptibility profile for all isolates of E. coli were merged into a single variable. F: Proportion of E. coli isolates resistant to one antimicrobial resistant to another antimicrobial are shown in the connections. The area covered by the connection on E is proportional to the level of co-resistance observed. Co-resistance proportions were scaled down to 1/15th of the actual overlap for visualization. Abbreviations: AMI: Amikacin, GEN: Gentamicin, TOB: Tobramycin, IMI: Imipenem, MER: Meropenem, CPL: Cephalexin, CPD: Cephradine, CFC: Cefaclor, CFU: Cefuroxime, CFT: Cefotaxime, CFD: Ceftazidime, CFN: Ceftriaxone, CFX: Cefixime, CPZ: Cefoperazone, CFP: Cefepime, CPZ-S: Cefoperazone-Sulbactam, PIP-T: Piperacillin-Tazobactam, AMX-C: Amoxicillin-Clavulanic acid, AMP-S: Ampicillin-Sulbactam, CIP: Ciprofloxacin, LEV: Levofloxacin, OFL: Ofloxacin, MOX: Moxifloxacin, AZT: Aztreonam, TRI-S: Trime-Sulphamethoxazole, and DOX: Doxycycline
Antimicrobial resistance in Acinetobacter A: Each section of the diagram represents the resistance observed in Acinetobacter species against the antibiotic. Size of each section is proportional to the proportion of Acinetobacter species resistant to the antibiotic over the study period. Antibiotics of the same class are shown in similar colors. B: Line graphs show temporal trends of proportion of resistant Acinetobacter species in a clockwise direction from 2011 to 2015. C: Bar charts show the comparison of susceptibility to resistant isolates in patients of different age groups. Moving from out to inward, bars represent proportion of resistant Acinetobacter species reported in children <5 years of age, young adults between
6 to 18 years, middle aged 19 to 45 years old, 45 to 65 years old patients, and elderly over 65 years of age, respectively. D: Gender-wise comparison to susceptibility to resistant Acinetobacter species is shown in form bars. Outer circle and inner circle shows proportion of resistant Acinetobacter species isolated from women vs. men, respectively. E: For co-resistance analysis, antibiotics belonging to the same class with same susceptibility profile for all isolates of E. coli were merged into a single variable. F: Proportion Acinetobacter species isolates resistant to one antimicrobial resistant to another antimicrobial are shown in the connections. The area covered by the connection on E is proportional to the level of co-resistance observed. Co-resistance proportions were scaled down to 1/10th of the actual overlap for visualization. Abbreviations: DOX: Doxycycline, TRI-S: Trime-Sulphamethoxazole, PIP-T: Piperacillin-Tazobactam, AMP-S: Ampicillin-Sulbactam, AMX-C: Amoxicillin-Clavulanic acid, CIP: Ciprofloxacin, LEV: Levofloxacin, OFL: Ofloxacin, MOX: Moxifloxacin, CFN: Ceftriaxone, CFD: Ceftazidime, CFT: Cefotaxime, CPZ: Cefoperazone, CFX: Cefixime, CFP: Cefepime, CPZ-S: Cefoperazone-Sulbactam, IMI: Imipenem, MER: Meropenem, TOB: Tobramycin, GEN: Gentamicin, and AMI: Amikacin.
Figure 4

Antimicrobial resistance in Salmonella enterica serovar Typhi (S. Typhi) A: Each section of the diagram represents the resistance observed in S. Typhi against the antibiotic. Size of each section is proportional to the proportion of S. Typhi resistant to the antibiotic over the study period. Antibiotics of the same class are shown in similar colors. B: Line graphs show
temporal trends of proportion of resistant S. Typhi in a clockwise direction from 2011 to 2015. C: Bar charts show the comparison of susceptibility to resistant isolates in patients of different age groups. Moving from out to inward, bars represent proportion of resistant S. Typhi reported in children <5 years of age, young adults between 6 to 18 years, and middle aged 19 to 45 years old, respectively. D: Gender-wise comparison to susceptibility to resistant S. Typhi is shown in form bars. Outer circle and inner circle shows proportion of resistant S. Typhi isolated from women vs. men, respectively. E: For co-resistance analysis, antibiotics belonging to the same class with same susceptibility profile for all isolates of E. coli were merged into a single variable. F: Proportion S. Typhi isolates resistant to one antimicrobial resistant to another antimicrobial are shown in the connections. The area covered by the connection on E is proportional to the level of co-resistance observed. Co-resistance proportions were scaled down to 1/10th of the actual overlap for visualization.

Abbreviations: CFN: Ceftriaxone, CFD: Ceftazidime, CFT: Cefotaxime, CPZ: Cefoperazone, CFX: Cefixime, CFP: Cefepime, CIP: Ciprofloxacin, LEV: Levofoxacin, OFL: Ofloxacin, MOX: Moxifloxacin, NAL-A: Nalidixic acid, AMP-S: Ampicillin-Sulbactam, AMX-C: Amoxicillin-Clavulanic acid, AMP: Ampicillin, AMX: Amoxicillin, and TRI-S: Trime-Sulphamethoxazole.
Antimicrobial resistance in Coagulase-negative Staphylococci (CoNS) A: Each section of the diagram represents the resistance observed in CoNS against the antibiotic. Size of each section is proportional to the proportion of CoNS resistant to the antibiotic over the study period. Antibiotics of the same class are shown in similar colors. B: Line graphs show temporal trends of proportion of resistant CoNS in a clockwise direction from 2011 to 2015. C: Bar charts show the comparison of susceptibility to resistant strains in patients of different age groups. Moving from out to inward, bars represent proportion of resistant CoNS reported in children <5 years of age, young adults between 6 to 18 years, middle
aged 19 to 45 years old, 45 to 65 years old patients, and elderly over 65 years of age, respectively. D: Gender-wise comparison to susceptibility to resistant CoNS is shown in form bars. Outer circle and inner circle shows proportion of resistant CoNS isolated from women vs. men, respectively. E: For co-resistance analysis, antibiotics belonging to the same class with same susceptibility profile for all isolates of CoNS were merged into a single variable. F: Proportion of CoNS isolates resistant to one antimicrobial resistant to another antimicrobial are shown in the connections. The area covered by the connection on E is proportional to the level of co-resistance observed. Co-resistance proportions were scaled down to 1/10th of the actual overlap for visualization. Abbreviations: AMI: Amikacin, GEN: Gentamicin, TOB: Tobramycin, AMP: Ampicillin, AMX: Amoxicillin, IMI: Imipenem, MER: Meropenem, CPL: Cephalexin, CPD: Cephradine, CFC: Cefaclor, CFU: Cefuroxime, CFT: Cefotaxime, CFD: Ceftazidime, CFN: Ceftriaxone, CFX: Cefixime, CPZ: Cefoperazone, CFP: Cefepime, AMX-C: Amoxicillin-Clavulanic acid, AMP-S: Ampicillin-Sulbactam, CIP: Ciprofloxacin, LEV: Levofloxacin, OFL: Ofloxacin, MOX: Moxifloxacin, FUS-A: Fusidic acid, CLI: Clindamycin, AZI: Azithromycin, ERY: Erythromycin, TRI-S: Trime-Sulphamethoxazole, and DOX: Doxycycline.
Figure 6

Antimicrobial resistance in Staphylococcus aureus (S. aureus) A: Each section of the diagram represents the resistance observed in S. aureus against the antibiotic. Size of each section is proportional to the proportion of S. aureus resistant to the antibiotic over the study period. Antibiotics of the same class are shown in similar colors. B: Line graphs show temporal trends of proportion of resistant S. aureus in a clockwise direction from 2011 to 2015. C: Bar charts show the comparison of susceptibility to resistant strains in patients of different age groups. Moving from out to inward, bars represent proportion of resistant S. aureus reported children in <5 years of age, young adults between 6 to 18 years, middle
aged 19 to 45 years old, 45 to 65 years old patients, and elderly over 65 years of age, respectively. D: Gender-wise comparison to susceptibility to resistant S. aureus is shown in form bars. Outer circle and inner circle shows proportion of resistant S. aureus isolated from women vs. men, respectively. E: For co-resistance analysis, antibiotics belonging to the same class with same susceptibility profile for all isolates of S. aureus were merged into a single variable. F: Proportion of S. aureus isolates resistant to one antimicrobial resistant to another antimicrobial are shown in the connections. The area covered by the connection on E is proportional to the level of co-resistance observed. Co-resistance proportions were scaled down to 1/10th of the actual overlap for visualization. Abbreviations: AMI: Amikacin, GEN: Gentamicin, TOB: Tobramycin, AMP: Ampicillin, AMX: Amoxicillin, IMI: Imipenem, MER: Meropenem, CPL: Cephalexin, CPD: Cephradine, CFC: Cefaclor, CFU: Cefuroxime, CFT: Cefotaxime, CFD: Ceftazidime, CFN: Ceftriaxone, CFX: Cefixime, CPZ: Cefoperazone, CFP: Cefepime, AMX-C: Amoxicillin-Clavulanic acid, AMP-S: Ampicillin-Sulbactam, CIP: Ciprofloxacin, LEV: Levofloxacin, OFL: Ofloxacin, MOX: Moxifloxacin, CLI: Clindamycin, AZI: Azithromycin, ERY: Erythromycin, TRI-S: Trime-Sulphamethoxazole, and DOX: Doxycycline.

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