

# Antimicrobial resistance patterns of WHO priority pathogens isolated in children from a tertiary care hospital in Southern India: A descriptive and time trend analysis

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## Research

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## Abstract

## Background

There is global consensus that Antimicrobial Resistance (AMR) poses an unprecedented challenge to modern medicine as we know it today; and the lack of new antibiotics in the pipeline is compounding the threat to contain emerging drug-resistant infections. In 2017, the World Health Organization (WHO) has articulated a priority pathogens list (PPL) to provide strategic direction to research and development of new anti-microbials. Anti-microbial resistance patterns of selected 'drug-bug' combinations based on the WHO-PPL in one tertiary health care facility in India are explored in this paper.

## Methods

Culture reports of laboratory specimens, collected between 1st January 2014 and 31st October 2019 from paediatric patients in a tertiary care hospital in India, were retrospectively extracted. The antimicrobial susceptibility patterns for selected antimicrobials based on the WHO-PPL are analysed and reported.

## Results

Of 12,256 culture specimens screened, 2,335 (19%) showed culture positivity; of which 1,556 were organisms from the WHO-PPL. *E. coli* was the most common organism isolated (37%) followed by *Staphylococcus aureus* (16%). Total 72% of *E. coli* were extended-spectrum beta-lactamases producers, 55% of Enterobacteriaceae were resistant to 3rd generation cephalosporins, and 53% of *Staphylococcus aureus* were Methicillin resistant. Time-trend analysis of the data showed continued high resistance to carbapenem in *E. coli*, *Klebsiella pneumoniae* and *Enterobacter cloacae*.

## Conclusions

The AMR trends and prevalence patterns are likely to be different, across various local settings, than as defined at the national level or the WHO-PPL. This difference needs to be recognised in decision and policy making. It is critical, that the evidence used at national and global levels, have reasonable geographical and population representation through standardised and more granular AMR surveillance, in order to improve the effectiveness of the overall national AMR response.

## Background

Antimicrobial resistance (AMR) has been recognised as a major threat to global health. According to the World Health Organization (WHO), microorganisms mutate when exposed to antimicrobials resulting in AMR, which consequently render medicines ineffective and infections to persist in the body, increasing the risk of spread to others<sup>1</sup>. Because of AMR, our ability to treat common pathogens becomes challenging, resulting in increased duration of illness, and rise in the number of complications and deaths. AMR impacts the healthcare system by increasing the duration of hospitalization and treatment, and the overall costs of health care. By 2050, an estimated 10 million deaths are projected to occur due to AMR<sup>2</sup>, while another study projected AMR to cost the global economy US\$100 trillion, in the same period<sup>3</sup>.

In 2015, the 68th World Health Assembly endorsed the Global Action Plan on Antimicrobial Resistance to tackle this global challenge<sup>4</sup>. This action plan has five strategic actions, focusing on: (1)improving awareness and understanding of AMR; (2) strengthening AMR surveillance; (3) reducing incidence of infections; (4) optimizing antimicrobial use; and (5) developing the economic case for AMR control. To support the Global Action Plan, WHO has developed a priority pathogens list (PPL), through a consultative process in 2016-17<sup>5</sup>. The prioritization process involved multi-criteria decision analysis (MCDA) which used information from multiple sources in a systematic manner, and included: disease mortality, transmissibility, treatability, preventability in health care settings, health care burden, preventability in community settings, etc. Tuberculosis as a disease was given the highest priority for research and development of new antimicrobials. Twelve families of drug resistant bacteria, posing the greatest threat to human health, were categorized as critical, high and medium priority organisms, in terms of their resistance to specific antimicrobials. Although this categorization was intended to prioritize and stimulate research and development of new antibiotics for specific drug-resistant, it also makes a call for the prevention of infection and the rational use of antibiotics in both humans and animals.

India is considered to be the highest consumer of antibiotics for human in the world<sup>6</sup>. The AMR situation in India has raised grave public health concern<sup>7</sup> and an action plan for its control is crucial.<sup>8,9</sup> Given its importance for human health, the Government of India has developed a National Action Plan on Antimicrobial Resistance (NAP-AMR) 2017-2021<sup>10</sup>. Strengthening the knowledge and evidence base through surveillance of AMR, is one of the five key strategies of this action plan. The Indian Council of Medical Research (ICMR) has established an Antimicrobial Resistance Surveillance & Research Network (AMRSN) across selected hospitals in India, focusing on drug resistance among six pathogens<sup>11</sup>. However, not many hospitals outside this network in India track AMR patterns among these pathogens. It is critical to understand the AMR patterns in tertiary care hospitals so that appropriate evidence is generated for decision making at various levels.

This study explores the AMR patterns for WHO priority pathogens identified in the Pediatrics Department of a tertiary care hospital in Mangalore, South India. The study site - the Department of Pediatrics, Yenepoya Medical College Hospital – refers around 2,000 culture tests of various clinical specimens annually, from outpatient and hospitalized cases. The results of the culture tests are mainly availed for treatment purposes, but are not systematically analyzed on a routine basis which could provide further evidence and guidance for the development of antimicrobial resistance control policy at the hospital and elsewhere.

We conducted a retrospective review of laboratory culture reports to profile antimicrobial susceptibility pattern of the WHO priority pathogens identified in clinical isolates from children visiting, or admitted to, the Paediatrics Department.

## Methods

Administrative permission to access laboratory culture records through the computer backbone system was obtained from Yenepoya Medical College. Retrospective culture reports between 1st January 2014 to 31st October 2019 from various clinical specimens were extracted from the computer backbone system. The sources and types of specimens collected for culture included blood, urine, stool, pus, cerebrospinal fluid (CSF), sputum and any other bodily fluids or clinical specimens such as catheter, umbilical, and central line tips. Culture reports of all paediatric cases were included, irrespective of the location of sample collection, namely: outpatients, inpatient wards, and neonatal and paediatric intensive care units (ICUs). Tests for antimicrobial susceptibility patterns were performed and the results uploaded to the computer backbone system by Microbiology Department. The Kirby-Bauer disk-diffusion method and/or by BD phoenix automated system were used for performing antimicrobial susceptibility patterns and reported according to standard (Clinical Laboratory Standards Institute - CLSI) guidelines.

The culture access numbers, of specimens for which any positive results were reported for any bacterial pathogens, were listed. The antibiogram (i.e., which antibiotics were tested, and which were susceptible or resistant) was extracted for all culture isolates. Culture access numbers were used to link culture source and the date of sample collection to the antibiograms. The laboratory reports indicating contamination were excluded at data entry. The reports containing duplicate or repeat samples, from same source and subject, were also excluded.

The data entry and analysis were performed on Microsoft Excel. The data from the computer backbone was entered directly on a master Excel sheet followed by removal of duplicates. De-identified data were organised by specimen types (such as blood, urine etc.) in chronological order. Number of bacterial pathogens isolated was summated by their species (such as *E. coli*) and resistance patterns. The pathogens were classified into three main groups (Critical Priority, High Priority and Medium Priority) for selected antimicrobials, based on WHO priority pathogens list (Fig. 1). The PPL defines priority of pathogens based on resistance to specific antimicrobials such as carbapenems, 3rd generation cephalosporins, vancomycin, methicillin, penicillins, or fluoroquinolones. Pathogens other than those in the WHO PPL were excluded from the analysis. Time trend graphs were prepared for some key pathogens.

## Ethical issues

This study did not involve human subjects directly. An approval from the scientific and ethics committees of Yenepoya Medical College was obtained for this study. To maintain confidentiality, no identifiable information such as names, addresses, or phone numbers of subjects were collected. The data set, once finalised, was delinked from culture access numbers before analysis, to retain confidentiality.

## Results

A total of 12,256 culture specimens were screened for bacteriological results, of which 2,335 (19%) showed culture positivity. Of these, 1,556 were from the set of WHO PPL organisms. The largest number of bacterial isolation was seen in urine specimens (50%) followed by blood (16%) (Table 1). *E. coli* was the most common organism isolated (576), followed by *Staphylococcus aureus* (252).

Table 1

World Health Organisation (WHO) Pathogen Priority List (PPL) organisms isolated in study site by specimen type from January 2014 to October 2019

Specimen	Blood	Urine	Stool	Pus	Sputum	CSF & sterile fluids	Aspirate	Swab	Tissue	Central lines and stents	Catheter Tip	ET tip	other instruments	Total
Critical Priority Organisms														
<i>Acinetobacter baumannii</i>	6	17	0	1	3	1	2	4	0	6	11	10	4	65
<i>Pseudomonas aeruginosa</i>	5	23	0	7	6	1	3	6	1	31	16	16	2	117
Enterobacteriaceae														
<i>E coli</i>	13	447	29	28	5	4	2	8	8	4	15	7	6	576
<i>Klebsiella spp</i>	35	124	4	7	11	2	2	7	1	10	13	13	0	229
<i>Enterobacter spp</i>	31	53	4	4	3	2	3	5	2	7	38	6	4	162
<i>Serratia spp</i>	0	17	0	1	1	0	1	0	0	1	5	3	1	30
<i>Proteus spp</i>	0	21	0	3	0	1	0	1	0	0	0	0	1	27
<i>Providencia spp</i>	0	5	0	0	0	0	0	0	0	0	0	0	0	5
<i>Citrobacter spp</i>	0	16	0	1	2	0	0	1	0	3	12	6	5	46
<i>Morganella spp</i>	0	8	0	0	0	0	0	0	0	0	0	0	0	8
<i>Others spp</i>	0	2	0	0	0	0	0	0	0	1	2	1	0	6
High Priority Organisms														
<i>Enterococcus faecium</i>	5	9	0	0	0	0	0	0	0	0	1	0	0	15
<i>Staphylococcus aureus</i>	135	12	0	66	2	0	1	26	0	2	2	4	2	252
<i>Salmonella species</i>	8	0	0	0	0	0	0	0	0	0	0	0	0	8
Medium Priority Organisms														
<i>Streptococcus pneumoniae</i>	3	0	0	0	1	0	0	2	0	0	0	0	0	6
<i>Shigella species</i>	0	1	3	0	0	0	0	0	0	0	0	0	0	4
<b>Total WHO PPL organisms</b>	<b>241</b>	<b>755</b>	<b>40</b>	<b>118</b>	<b>34</b>	<b>11</b>	<b>14</b>	<b>60</b>	<b>12</b>	<b>65</b>	<b>115</b>	<b>66</b>	<b>25</b>	<b>1556</b>

The drug resistance among WHO PPL organisms was found to be highest among *E. coli*, *Klebsiella 'spp'*, and *Staphylococcus aureus* (Table 2). Nearly half of *Enterobacteriaceae* were resistant to Carbapenem and 3rd generation cephalosporins. About 72% of *E. coli* were extended-spectrum beta-lactamase (ESBL) producers. Similarly, half of *Staphylococcus aureus* were Methicillin resistant.

Table 2  
Specified antimicrobial resistance in World Health Organisation (WHO) Pathogen Priority List (PPL) organisms isolated in the study from January 2014 to October 2019

Critical Priority Organisms	Carbapenem-resistant			3 rd generation cephalosporin-resistant		
	Tested	Resistant	Percentage	Tested	Resistant	Percentage
<i>Acinetobacter baumannii</i>	52	6	11.54%			
<i>Pseudomonas aeruginosa</i>	59	3	5.08%			
<b>Enterobacteriaceae</b>	<b>606</b>	<b>276</b>	<b>45.54%</b>	<b>721</b>	<b>396</b>	<b>54.92%</b>
<i>E coli</i>	342	140	40.94%	410	297	72.44%
<i>Klebsiella spp</i>	107	58	54.21%	133	84	63.16%
<i>Enterobacter spp</i>	103	59	57.28%	111	8	7.21%
<i>Serratia spp</i>	10	9	90.00%	10	2	20.00%
<i>Proteus spp</i>	15	1	6.67%	20	0	0.00%
<i>Providencia spp</i>	4	1	25.00%	5	0	0.00%
Citrobacter spp	13	6	46.15%	19	4	21.05%
Morganella spp.	6	0	0.00%	7	1	14.29%
<i>Others spp</i>	6	2	33.33%	6	0	0.00%
High Priority Organisms	vancomycin-resistant			Methicillin resistant <i>Staphylococcus aureus</i> (MRSA)		
	Tested	Resistant	Percentage	Tested	Resistant	Percentage
<i>Enterococcus faecium</i> ,	15	2	13.33%			
<i>Staphylococcus aureus</i>	174	3	1.72%	188	99	52.66%
	fluoroquinolone-resistant					
	Tested	Resistant	Percentage			
<i>Salmonella species</i>	4	3	75.00%			
Medium Priority Organisms	fluoroquinolone-resistant			Penicillin-resistant		
	Tested	Resistant	Percentage	Tested	Resistant	Percentage
<i>Streptococcus pneumoniae</i>				6	2	33.33%
<i>Shigella species</i>	4	3	75.00%			

Time trend analysis over the past four years, for selected WHO 'Critical priority' pathogens, continues to show high resistance for carbapenem in *E coli*, *Klebsiella pneumoniae* and *Enterobacter cloacae* (Fig. 2). Similarly, *E coli*, *Klebsiella pneumoniae* continue to show trends of high ESBL rates (Fig. 3). Among the WHO 'High priority' pathogens, *Staphylococcus aureus* continues to show high methicillin-resistance in recent years (Fig. 4).

Three of the WHO 'High priority' pathogens namely, *Helicobacter pylori* (clarithromycin resistant), *Campylobacter species* (fluoroquinolone-resistant) and *Neisseria gonorrhoeae* (3rd generation cephalosporin-resistant, fluoroquinolone-resistant), were not detected in our study specimens. Similarly, one of the WHO 'Medium priority' pathogen, *Haemophilus influenzae* (ampicillin-resistant), was not observed in this study.

## Discussion

The 2017 guidance document of WHO indicated the highest carbapenem resistance worldwide in *Acinetobacter baumannii* (91%) and *Pseudomonas aeruginosa* (82%), which is one of the reasons for classifying them as Critical Priority<sup>5</sup> pathogens. The same study reported > 50% carbapenem resistance in *Acinetobacter baumannii*, and 31–50% carbapenem resistance in *Pseudomonas aeruginosa* in the Indian sub-continent. Early results from the surveillance data from up to 22 ICMR-AMRSN sites in India showed around 80% carbapenem resistance in *Acinetobacter baumannii* and around 30% in *Pseudomonas aeruginosa*<sup>11</sup>. However, another study in children from Mumbai, India, identified only 15% of *Pseudomonas aeruginosa* are resistant to carbapenem<sup>12</sup> which is comparable to our study. This observation of low AMR in our study is based on less than 60 specimens in one tertiary hospital, and need not be considered representative of the situation in India. On the other hand, the variable incidence across study sites indicate carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, may perhaps be not that high across India.

The WHO report<sup>5</sup> identified high carbapenem resistance in *E. coli* (55%), *Klebsiella* (70%) and *Enterobacter 'spp'* (59%), which is comparable to the findings in this study. The ICMR-AMRSN data also showed similar carbapenem resistance prevalence in *Klebsiella pneumoniae* (40%- 50%) but a significantly lower level in *E.coli* (15–25%). The ESBL trends in *E. coli* and *Klebsiella 'spp'* (70–80%) as well as methicillin-resistance in *Staphylococcus aureus* (53%), were high in this

study and comparable to the WHO report. However, due to the low sample size of *Salmonella spp*, *Shigella spp* and *Streptococcus pneumoniae*, it may not be appropriate to compare the results from this study to others.

Several studies on AMR have been published in India in recent years. However, the data on AMR patterns in systematically collected clinical specimens from multiple sources is limited. Nevertheless, it shows high variability across study sites and settings. An observational study from a tertiary care hospital in Puducherry showed 12.5% of isolated organisms exhibited resistance to one or more drugs, but did not focus the results on WHO PPL pathogens<sup>13</sup>. Another retrospective five year follow-up study in a tertiary care hospital in North India showed increasing trends of AMR in urinary tract infection-causing isolates<sup>14</sup>. This study reported prevalence of ESBL increased from 22% in 2009 to 33% in 2014, which is a lot lower than our study. Increasing trends of AMR was observed among gram negative samples collected across seven hospitals in India over a four-year period, but the reported carbapenem resistance prevalence in *Klebsiella 'spp'* (39%) and *E. coli* (12%) were lower than our study<sup>15</sup>. Another observational study among intensive care unit (ICU) patients in a tertiary care hospital in Delhi showed 80% of *Klebsiella 'spp'* were resistant to beta-lactam group of antimicrobials, which is similar to our study<sup>16</sup>. A retrospective data collected for one year in the ICU of a hospital in Southern India found that 40.6% of *Staphylococcus aureus* were Methicillin-resistant (MRSA) and 11.9% of *Enterococci* were Vancomycin-resistant which is comparable to the current study<sup>17</sup>. Among *Enterobacteriaceae* isolated from a paediatric tertiary care hospital in Mumbai, 24% were extended spectrum beta-lactamase (ESBL) producers and 27% were carbapenem-resistant isolates showing a lower resistance level than our study<sup>12</sup>.

As our study has deployed retrospective data collection based on computerised laboratory records, it has several limitations, but which may have been partially compensated due to the large sample size and standardised quality checks within the hospital. The sample collection, microbiological analysis, and report update on computerised records were done on a routine basis alongside the provision of healthcare services. Although the health facility maintains good quality of clinical and laboratory services along with proper documentation, one cannot ensure that the quality checks in retrospective data is fully compatible with the highest quality standards of a well-conducted prospective study. Although the sample collection, microbiological analysis, and report updates uses standard procedures, it has likely been conducted by different people over the 5 year-period, which may have had inter-personnel variations on the quality of laboratory procedures.

There were no standard inclusion criteria for sample collection; as it was generally left to the discretion of the treating physician. Furthermore, culture methodology and practices may have changed over time during the (period under study due to various operational reasons. Occasional shortfall of laboratory reagents and other essential supplies for conducting tests may have resulted in the omission of some tests during certain periods, resulting in some bias, the direction of which is difficult to assess. Some of the WHO PPL pathogens were not included in this study as culture specimens were collected from children and which do not generally include genitourinary swabs or gastric biopsy specimens suitable for the culture of *Neisseria gonorrhoeae* or *Helicobacter pylori*. Similarly, *Campylobacter 'spp'* was also not identified in the specimens either because of low incidence or because samples collected may not be best suited for its isolation. However, the large sample size was an important strength of this study.

## Conclusion

Among the WHO PPL pathogens, *E. coli*, *Klebsiella* species, *Enterobacteriaceae* and *Staphylococcus aureus* (methicillin-resistant) seem to have high AMR patterns in our study site. On the other hand, AMR patterns for *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (vancomycin resistant) seem to be lower than the WHO global estimates. This study highlights the wide variability of AMR prevalence patterns across sites *within* countries, leave alone *across* countries. National and global AMR trends, using surveillance in tertiary hospitals as the basis for trend analysis, may not accurately reflect the trends and prevalence patterns within local milieus. It is imperative for policy decisions to factor in geographical and population representativeness within AMR surveillance networks so that national or global action plans are implemented based on a reasonable evidence base if not a robust one. While AMR policies, action plans and control activities should be aligned with global and national trends, they should be adaptive to the need and variability of local situations. It is essential that large health facilities carefully monitor and review emerging AMR patterns and trends periodically so that they can prioritise, plan, and implement health facility level policies and guidelines for the optimal use of antimicrobials.

## Declarations

## Ethics approval and consent to participate

Described under manuscript

## Consent for publication

All authors have consented for publication.

## Availability of data and material

All the data included in the manuscript.

## Competing interests

## Funding

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## Authors' Contributions

VVM served as Principal Investigator for this study and took overall responsibility for protocol development, study design, ethics approval, data collection, analysis, and drafted the manuscript. VM conceptualized the study design, provided guidance on data extraction, analysis and edited the manuscript. PS, VS and RPD provided institutional support in data collection, expert advice, and contributed to the manuscript. All authors have approved the final version of the manuscript.

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## Figures

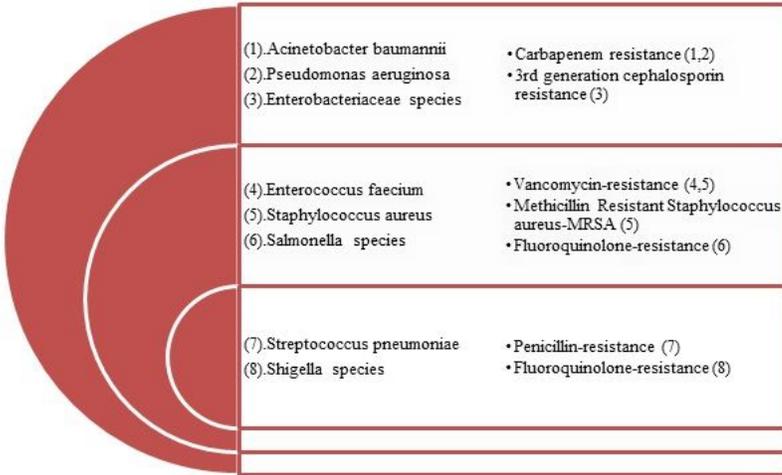


Figure 1

The list of organisms and antimicrobial resistance patterns included in the analysis based on World Health Organization priority pathogens list (PPL)

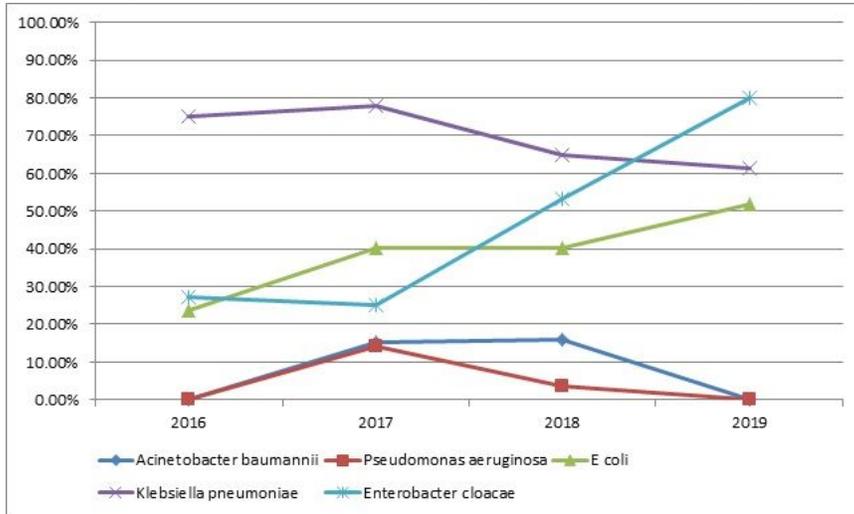


Figure 2

Carbapenem-resistance trends among selected WHO Critical priority pathogens

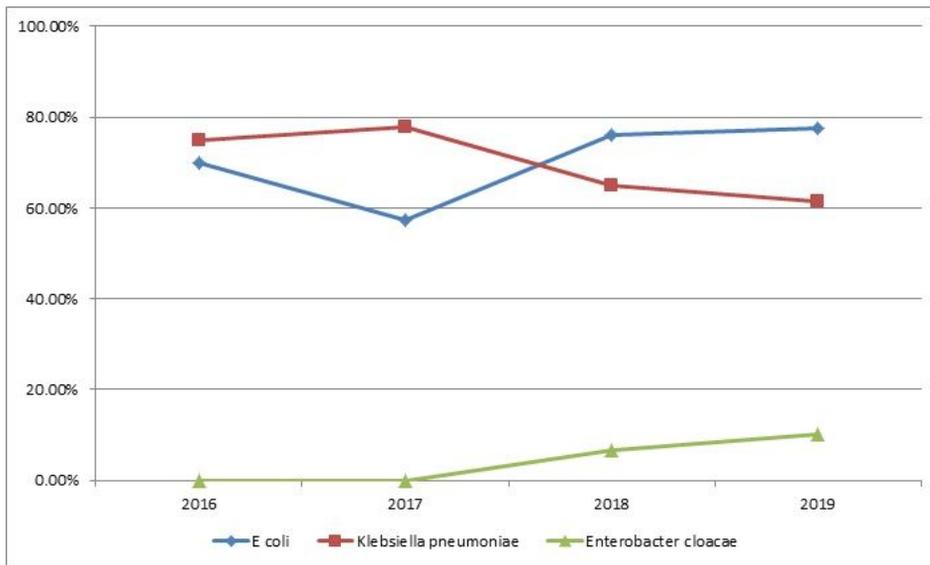


Figure 3

Third generation cephalosporin-resistant trends (extended-spectrum beta-lactamases (ESBL) producers) among selected WHO Critical priority pathogens

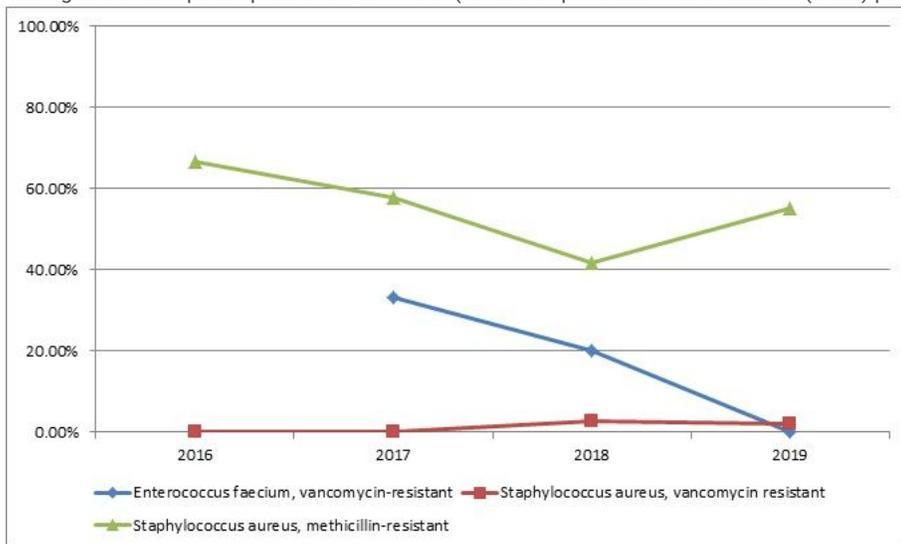


Figure 4

Resistance trends among selected WHO High Priority pathogens