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Five-Year Resistance Trends Of Bacterial Isolates In Kigali, Rwanda

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Abstract

Antimicrobial resistance (AMR), the phenomenon of decreased sensitivity to pharmaceutical agents that kill or inhibit the growth of bacterial pathogens, is a serious threat to public health worldwide. Many countries, including Rwanda, lack current information on antibiotic sensitivity profiles that would greatly aide clinicians and policy-makers. The current study aims to describe the time trends of antibiotic sensitivity for the isolates of six different bacterial pathogens from patients at King Faisal Hospital in Kigali, Rwanda and to make inference about future patterns of drug resistance in the study setting, to guide physicians in their prescribing practices and provide baseline data for future interventions. This was a retrospective observational study that involved data collection of the frequency and antimicrobial sensitivity patterns of bacterial organisms isolated from cultures of clinical specimens collected from patients at the King Faisal Hospital in Kigali, Rwanda. Data were collected over a five-year period from January 1, 2009 to December 31, 2013. Cochran-Armitage test and Somers' D statistic were used to determine trends in antibiotic sensitivity over time. Analysis found that the majority of isolates collected over the study period were of E. coli (46.7%). Amongst the gram-negative species, Colistin consistently elicited the highest average annual antibiotic sensitivity. Notably, Acinetobacter spp. showed the greatest resistance to all antibiotics, relative to other species of its group. Vancomycin showed the greatest activity against gram-positive bacteria. Trend analysis determined that Imipenem and Pipercillin demonstrated negative annual sensitivity trends more often than any other antibiotics. AMR trends showed that decreased bacterial sensitivity to Imipenem and Pipercillin is increasing over time and limits the usefulness of the drugs for empiric therapy of gram infections.

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Introduction:

Antimicrobial resistance is the phenomenon of decreased sensitivity to pharmaceutical agents that kill or inhibit the growth of bacterial pathogens [1]. It is becoming increasingly problematic as the proportion of pathogens, including *Staphylococcus* spp., *Klebsiella* spp., and *E. coli*, continues to increase relative to those which are still drug sensitive. Rapid emergence of antimicrobial resistance (AMR) seriously threatens the progress we have made in decreasing morbidity and mortality from many pathogens with the use of antibiotics, and is a pressing public health concern worldwide, in upper-, middle-, and low-income nations alike [1,2]. Although AMR is not a new phenomenon, it is made more severe by the lack of new antibiotic agents being produced, and is particularly problematic in low- and middle-income countries. In addition to still battling infectious disease, these countries also face limited access and financial ability to buy newer, more effective antimicrobials [2,3].

Many countries, including Rwanda, lack current information on antibiotic susceptibility profiles that would greatly aide clinicians and policy-makers. A review of available studies on this topic in Eastern Africa show that there is an increasing availability of publications from the mid-1970's to the present, but that most reported research was conducted in Kenya and Ethiopia, with less (but still an increasing amount over time) from Uganda and Tanzania, and the least from Rwanda and Burundi [2]. Omulo et al. even go as far as to say that there is a general low prioritization of AMR in Sub-Saharan Africa, and that even in countries with higher number of publications like Kenya, the progress of research is slow relative to the global awareness of AMR in enteric bacteria [2]. The paucity of available data on trends of antibiotic sensitivity in Rwanda creates a need for descriptive studies on the landscape of AMR in this country.

Available published studies about AMR in Rwanda indicate the presence of antibiotic resistant strains of a number of different pathogens, including *Streptococcus*, *Neisseria*, and *Shigella* in the late 1980s and early 1990s. In a 1993 study of 383 clinical isolates of *Streptococcus pneumoniae* in Kigali, Rwanda, researchers found that 2I % all isolates were confirmed as relatively resistant *S. pneumoniae*, called RRSP [4]. Specimens were collected between 1984-1990 from 230 children and 153 adults at the Centre Hospitalier de Kigali with community-acquired infection. Isolates did not show a high level of resistance to penicillin G, but did exhibit resistance to chloramphenicol (3I % RRSP) and 6 % resistance in the penicillin susceptible strains (PSSP) [4]. Both RRSP and PSSP strains demonstrated doxycycline resistance, and all isolates remained fully susceptible to erythromycin. This study implied that penicillin G, ampicillin and chloramphenicol should not be used alone as empirical treatment for pneumococcal meningitis in patients in Rwanda [4]. Further information on drug classes and mechanisms of action and resistance is provided in the appendix.

In Kigali specifically a study of antimicrobial resistance trends of *Neisseria gonorrhea* conducted from 1985-1993 tracked changes in antibiotic sensitivity for various drugs. Specimens were collected from men, women, and pre-pubertal girls reporting genital symptoms at the Centre Hospitalier de Kigali or the Centre Médico Social de Bilyogo. They found an increase in penicillinase-producing *N. gonorrheae* over the study period: these bacteria accounted for 39% during 1985-1991, but rose to 61% in 1992-1993 [5]. They also identified plasmid-mediated resistance to tetracycline (TRNG) for the first time at the end of 1989, which increased from 2% of the isolates in 1990 to 50% by 1993 [5]. Overall, resistance to penicillin, thiamphenicol, and tetracycline was common in *N. gonorrheae* during 1985–1993 [5]. Another similar study was conducted on trends of *N. gonorrhoeae* resistance between 1986-2000, and found that while all gonococcal isolates were susceptible to ceftriaxone, ciprofloxacin, and spectinomycin (the recommended drugs of WHO), there were increasing levels of plasmid-mediated resistance in *N. gonorrhoeae* as well as chromosomal-mediated resistance [6]. Between April 1999-April 2000 samples were obtained from men with urethral syndromes and neonates with purulent conjunctivitis from the Centre Médico Social in Bilyogo, Rwanda. The results underscore that resistance levels to thiamphenicol and sulfamethoxazole/trimethoprim vary from country to country, and these drugs should not be

recommended without a reliable baseline susceptibility assessment, clinical trials, and regular surveillance [6]. A 1997 study on AMR of *Shigella* isolates in Kigali from 1983-1993 found that there was increasing frequency of resistance to multiple antibiotics [7]. They found that resistance to tetracycline was common in all *Shigella* species, and remained unchanged over time. However, they also documented increasing resistance to ampicillin, chloramphenicol, and trimethoprim among endemic *Shigella* spp., and increasing resistance to trimethoprim and nalidixic acid among *S. dysenteriae* Type 1 isolates [7]. All isolates remained fully susceptible to norfloxacin and ciprofloxacin, but importantly, they identified several cases of combined AMR: 68% of *S. flexneri*, 78% of *S. sonnei*, 99% of *S. dysenteriae* Type 1 and 65% of *S. boydii* / *S. dysenteriae* Types 2-10 isolates that were resistant to more than one antibiotic [7]. This evidence emphasized the problem of multi-resistant *Shigella* isolates in Rwanda, and the need for consistent, continued monitoring of AMR and serotype distribution.

A more recent study, from Muvunyi et al. 2011, provided further evidence that there is increasing resistance, and that continued use of first-line treatments may be contributing to resistance [8]. Samples from both inpatients and outpatients with urinary tract infections were collected from Butare University Hospital and Kigali University Hospital. The most common isolate from over 1,000 urine cultures was *E. coli*. (60.7%), and it was found that the antibiotics commonly used in UTIs exhibit decreasing efficacy except Fosfomycin-trometamol and imipinem [8]. Risk factors associated with ciprofloxacin-resistant *E. coli* included use of ciprofloxacin or other antibiotics in the previous 6 months, and production of ESBL. Risk factors for ESBL positivity included the use of ciprofloxacin and third-generation cephalosporin in the previous 6 months and being an inpatient [8]. These results suggest that alternatives are needed to address urinary tract infection in Rwanda, with Fosfomycin-trometamol proposed by the authors as a possible suitable therapy option.

The most recent publication available, by Ogbuagu et al. (2015) documents high prevalence of AMR in Rwanda[3]. It was conducted to determine and describe the prevalence of AMR among pathogens associated with common infections in patients on the wards of the largest tertiary hospital in Rwanda. The study evaluated antibiotic sensitivity patterns of bacterial pathogens cultured from urine, blood, sputum, and wound swab specimens obtained over a 6-month period, and found that 31.4% and 58.7% of *Escherichia coli* and *Klebsiella* isolates, respectively, were resistant to at least one of the third generation cephalosporins[3]. Eight percent of *E. coli* isolates were resistant to imipenem; 82% and 6% of *Staphylococcus aureus* strains were oxacillin- and vancomycin-resistant-respectively[3].

Moving forward, it will be important to further classify the patterns in microbial resistance in Rwanda, in order to illustrate the landscape of AMR threats so that researchers, physicians, and policy makers may be better informed. The current study describes the time trends of antibiotic sensitivity for six different bacterial pathogens isolated from patients at King Faisal Hospital in Kigali, Rwanda. It also seeks to make inference about future patterns of drug resistance in the study setting, to guide physicians in their prescribing practices and provide baseline data for future interventions.

Methods:

Study Design

This was a longitudinal study of the bacterial specimens (n=5296) collected by the internal medicine department at the King Faisal Hospital in Kigali, Rwanda, from January 1, 2009 to December 31, 2013. Data was collected on the frequency and antimicrobial sensitivity patterns of all cultures. Samples with improper labeling and those with inadequate patient and specimen identifiers were excluded from the sample.

Sample collection and processing

Clinical specimens included urine, blood, sputum, and pus swab specimens from adult patients on internal medicine wards. Blood samples were collected and incubated into BD Bactec culture vials.

Urine, wound, and sputum cultures were collected in sterile containers. Laboratory materials, including sterile containers, antibiotic disks, and culture media, were obtained from Beckton, Dickinson, and Company (NJ).

Blood samples were directly incubated in the BACTEC 9050 at 37°C for 5 days, and cultures were assessed daily for growth or presence of pathogens. Samples with bacterial growth were sub-cultured on appropriate media guided by gram stain results as follows: gram-positive cocci were plated on mannitol salt agar (MSA) and blood agar, whereas MacConkey agar and blood agar media were used for isolation of gram-negative bacilli. Additional identification of gram-positive cocci species was performed using catalase and coagulase tests. Identification of species of gram-negative bacilli was done by colony morphology and by using API 20 E strips (bioMerieux).

Urine samples, after wet mount examination, were cultured on blood agar and cysteine lactose electrolyte-deficient (CLED). The number of colonies was counted after 18–24 hours of incubation at 37°C. Specimens with > 10^5 CFU/mL urine were considered significant. Maximum duration of incubation was 48 hours. For wound swabs and sputum specimens, the gram stain morphology of principal pathogens dictated the selection of appropriate medium for culture, which was then incubated at 37°C for 24 hours. As with other specimens, identification of bacterial species was done using a combination of colony morphology, growth characteristics on selective media and by using API 20 E strips (bioMerieux) for Gram negative bacilli.

Antibiotic susceptibility testing was performed by the Kirby Bauer disk diffusion method. The following antibiotic disks were used: ampicillin, 10 mg; ceftazidime, 30 mg; cefotaxime, 30 mg; ceftriaxone, 30 mg; cefalothin, 30 mg; cefuroxime, 30 mg; ciprofloxacin, 5 mg; amikacin, 30 mg; amoxicillin/clavulanic acid (amox/clav), 20/10 mg; erythromycin, 10 mg; gentamicin, 10 mg; imipenem, 10 mg; norfloxacin, 10 mg; penicillin, 10 units; oxacillin, 1 mg; piperacillin, 100 mg; and vancomycin, 30 mg.

A suspension from growth on solid media plates was prepared by adding bacterial colonies into sterile distilled water until it approximated the same turbidity as the MacFarland turbidity standard 0.5. The resulting suspension was inoculated on Muller Hinton agar by using a sterile cotton swab. After this procedure, the antibiotic disks were added to the plate with at least 20 mm between each disk and subsequently incubated at 37°C for 18–24 hours; thereafter, interpretation of the diameter of inhibition was done according to 2012 Clinical and Laboratory Standards Institute (CLSI) guidelines. Quality control for the Kirby Bauer disk diffusion test was performed using three American Type Culture Collection (ATCC) strains: *Escherichia coli* ATCC 25922, *S. aureus* ATCC 25923, and *Pseudomonas* spp. ATCC 27853. Suspensions of the organisms were prepared as described above, and the inhibition diameter obtained was compared with the standard range expected for the ATCC strains.

Data format: Data was aggregated annually, so that the proportion of bacteria from each source (urine, blood, sputum, and pus) susceptible to each drug was provided for each year.

Statistical Analysis:

Multiple approaches for identifying temporal trends were explored, although data format made the Mann-Kendall test and Wilcoxon rank-sum test inappropriate. Ultimately, the Cochran-Armitage Trend Test and the Somers' D Test [9] were used to test for trends in AMR across the 5 years. The "EXACT" statement of the Cochran-Armitage command produces exact p-values for the Cochran-Armitage test, indicating the probability that the dependent variable decreases as the independent variable increases, or that the dependent variable increases as the independent variable does. Also included in the output

is the Somers' D(R|C), which measures the association treating the dependent (row) variable as the response and the independent (column) variable as a predictor. If the asymptotic 95% confidence limits do not contain 0, this indicates strong association (positive trend if the values are both greater than 0, and negative trend if the values are both less than 0).

Results:

Description of sample- Of all 5296 isolates collected between 2009 and 2013, 46.70% were of *E. coli*, 18.41% were of *Klebsiella* spp., 5.91% were of *Acinetobacter* spp., 7.08% were of Pseudomonas spp., 11.65% were of *S. aureus*, and 10.25% were of *Enterococcus* spp. (see Table 1).

E. coli was found to be most sensitive to Colistin (98.6% ± 2.0), Imipenem (92.2% ± 11.0), and Nitrofurontoin (84.8% ± 3.7) over the course of 2009-2013. It was least sensitive to Ampicillin (14.8 ± 3.7), Piperacillin (35.4 ± 10.1), and Amoxicillin+Clavulanate (36.0 ± 21.7), which are all Aminopenicillin drugs. However, there was large variation in sensitivity figures for Piperacillin and Amoxicillin. For further information about sensitivity trends for *E. coli*, see Supplemental Table 1a.

Klebsiella spp. was tested by a majority of the antibiotics (16/23). It was found to be most sensitive to Colistin (99.8% ± 1.8), Imipenem (89.4% ± 10.8), and Norfloxacin (69.8% ± 14.3) over the study period. It was least sensitive to Piperacillin (18.2 ± 4.0), Amoxicillin+Clavulanate (24.6 ± 14.3), and Ceftriaxone (24.8 ± 18.9), all of which are ß-Lactam antibiotics. Further information about sensitivity trends for *Klebsiella* spp. can be found in Supplemental Table 1b.

Acinetobacter spp. was most sensitive to Colistin (81.5% ± 20.2), Amikacin (59.2% ± 22.4), and Imipenem (45.2% ± 29.0). Notably, this species exhibited some of the lowest average sensitivity values, as only 7.4% of *Acinetobacter* isolates from 2009-13 were sensitive to Ceftriaxone, and only 8.0% were sensitive to Cefotaxime, as compared to 24.8% and 29.0% in *Klebsiella* spp., respectively. This species was the least sensitive to Ceftriaxone (7.4 ± 4.0), Cefotaxime (8.0 ± 3.3), and Ceftazidime (15.4 ± 4.0), all Cephalosporin drugs. Further information about sensitivity trends for *Acinetobacter* spp. can be found in Supplemental Table 1c.

Pseudomonas spp. was most sensitive to Colistin (97.0% ± 6.0), Imipenem (84.3% ± 31.5), and Ciprofloxacin (82.8 ± 4.6). Compared to the other species, *Pseudomonas* spp. exhibited relatively high levels of sensitivity—the lowest level of sensitivity was to Cefotaxime (49.4 ± 20.4), and this had a large standard deviation. Overall, this species showed low sensitivity to Cefotaxime, Pipercillin (77.0 ± 6.5), and Gentamicin (77.0 ± 9.4). Further information about sensitivity trends for *Pseudomonas* spp. can be found in Supplemental Table 1d.

S. aureus was most sensitive to Vancomycin (100% all five years), Oxacillin (97.8% ± 1.10), and Gentamicin (87.2% ± 6.46). This pathogen showed relatively high levels of sensitivity, as only 3 of the 9 antibiotic groups tested showed sensitivity percentages of less than 70% during the period 2009-13. It was least sensitive to Ampicillin (20.0 ± 6.3), Amoxicillin+Clavulanate (40.6 ± 23.0), and Erythromycin (64.0 ± 10.7). Further information about sensitivity trends for *S. aureus* can be found in Supplemental Table 1e.

Enterococcus spp. shows greatest susceptibility to Vancomycin (99.4± 1.3), Amoxicillin + Clavulanate (89.0%± 6.2), and Ampicillin (82.6% ± 1.8) during the years 2009-13. This species showed the greatest sensitivity to Ampicillin and to Amoxicillin of any of the included species. During the five-year period, it was least sensitive to Penicillin (26.8 ± 12.7), Gentamicin (27.0% ± 6.0), and Levofloxacin (54.6 ± 18.4).

Trend Determination- The results of the Cochran-Armitage and Somers' D tests are shown in Figure 1. The majority of the pathogens (4 of 6) were not tested with Erythromycin, Cephalexin, Oxacillin, Vancomycin, or Penicillin. When annual bacterial susceptibility testing did occur for a pathogen, the most likely pattern of sensitivity across the five year period was 'No Trend' (41/61= 67.21%). Of the remaining 20 groups, the majority (17/20) were characterized as 'Negative Trend', which indicates decreasing antibiotic sensitivity, or increasing AMR. Only 3 groups were classified as having a 'Positive Trend': *Acinetobacter* spp./Colistin, *S. aureus*/Erythromycin, and *Enterococcus* spp./Vancomycin.

Across the six bacterial groups, *E. coli* has the greatest number of 'Negative Trend' groups (n=5), followed by *Klebsiella* spp. and *Acinetobacter* spp., with each having 4 'Negative Trend' groups. E. coli had decreasing sensitivity, or increasing resistance, to Gentamicin, Nalidixic Acid, Pipercillin, Imipenem, and Colistin. There was no trend for Ampicillin, Amoxicillin+Clavulanate, Norfloxacin, Ciprofloxacin, Cefuroxime, Cotrimoxazole, or Nitrofurantoin. Figure 2a depicts the five antibiotics that had negative trends over time for *E. coli*. Figure 2b depicts *E. coli* sensitivity response to all antibiotics.

Klebsiella spp. had 4 'Negative Trend' groups, indicating decreasing sensitivity to Amoxicillin+Clavulanate, Ciprofloxacin, Pipercillin, and Imipenem. There was no trend for Gentamicin, Nalidixic Acid, Norfloxacin, Cefuroxime, Cotrimoxazole Nitrofurantoin, Amikacin, Cefotaxime, Ceftriaxone, Levofloxacin, Ceftazidime, or Colistin. Figure 3a depicts the four antibiotics that had negative trends over time for *Klebsiella* spp. Figure 3b depicts *Klebsiella* spp. response to all antibiotics.

Acinetobacter spp. also had 4 'Negative Trend' groups, indicating decreasing sensitivity to Ciprofloxacin, Ceftriaxone, Levofloxacin, and Imipenem. There was no trend for Amikacin, Cefataxime, or Ceftazidime. Figure 4a depicts the four antibiotics that had negative trends over time for *Acinetobacter* spp. Figure 4b. depicts *Acinetobacter* spp. response to all antibiotics.

Importantly, Imipenem can be seen as a recurring entity in each of Figures 2a, 3a, and 4a, indicating that it is decreasing in efficacy yearly against multiple bacterial pathogens, and requires further study. Amongst the group of 23 antibiotics tested against the different bacterial species, Piperacillin and Imipenem registered the greatest frequency of 'Negative Trend' indications. Plotting the average susceptibility proportion for all isolates in each year for Imipenem and Piperacillin yields a graph depicting negative trends over time for Imipenem and Piperacillin, respectively, as can be seen in Figure 5.

Results:

Table 1. Average annual antimicrobial sensitivity (%) and corresponding standard deviation of all isolates collected from King Faisal Hospital between 2009-2013.

	Bacterial species					
Antimicrobial Agent	*Escherichia coli* (n= 2473)	*Klebsiella* spp. (n= 975)	*Acinetobacter* spp. (n=313)	*Pseudomonas* spp. (n= 375)	*Staphylococcus aureus* (n= 617)	*Enterococcus* spp. (n= 543)
Ampicillin	14.8 (3.7)	N/A	N/A	N/A	20.0 (6.3)	82.6 (1.8)
Amoxicillin +Clavulanate	36.0 (21.7)	24.6 (14.3)	N/A	N/A	40.6 (23.0)	89.0 (6.2)
Gentamicin	74.0 (2.6)	51.8 (3.8)	N/A	77.0 (9.4)	87.2 (6.5)	27.0 (6.0)
Nalidixic Acid	51.6 (5.1)	54.6 (12.0)	N/A	N/A	N/A	N/A
Norfloxacin[a]	66.4 (2.9)	69.8 (14.3)	N/A	N/A	N/A	N/A
Ciprofloxacin	50.8 (9.7)	49.6 (5.0)	18.4 (1.8)	82.8 (4.6)	85.0 (9.7)	N/A
Cefuroxime	75.2 (6.0)	48.4 (17.2)	N/A	N/A	N/A	N/A
Piperacillin	35.4 (10.1)	18.2 (4.0)	N/A	77.0 (6.5)	N/A	70.4 (32.2)
Cotrimoxazole	28.5 (3.4)	28.4 (6.2)	N/A	N/A	72.0 (15.8)	N/A
Nitrofurontoin[a]	84.8 (3.7)	47.8 (8.6)	N/A	N/A	N/A	N/A
Amikacin	N/A	64.8 (13.4)	59.2 (22.4)	76.8 (16.3)	N/A	N/A
Cefotaxime	N/A	29.0 (8.7)	8.0 (3.3)	49.4 (20.4)	N/A	N/A
Ceftriaxone	N/A	24.8 (18.9)	7.4 (4.0)	N/A	N/A	N/A
Levofloxacin	N/A	46.0 (0.0)[b]	22.6 (12.7)	N/A	N/A	54.6 (18.4)
Ceftazidime	N/A	31.6 (12.5)	15.4 (4.0)	81.0 (11.4)	N/A	N/A
Imipenem	92.2 (11.0)	89.4 (10.8)	45.2 (29.5)	84.3 (31.5)[b]	N/A	N/A
Chloramphenicol	N/A	N/A	N/A	N/A	N/A	59.8 (10.6)
Colistin	98.6 (2.0)	99.8 (1.8)	81.5 (20.2)[b]	97.0 (6.0)[b]	N/A	N/A
Erythromycin	N/A	N/A	N/A	N/A	64.0 (10.7)	N/A
Cephalexin	N/A	N/A	N/A	N/A	86.4 (5.7)	N/A
Oxacillin	N/A	N/A	N/A	N/A	97.8 (1.1)	N/A
Vancomycin	N/A	N/A	N/A	N/A	100 (0.0)	99.4 (1.3)
Penicillin	N/A	N/A	N/A	N/A	N/A	26.8 (12.8)[b]

[a] For urine isolates only

[b] Less than 5 years of data; only groups with at least 3 years were included.

N/A: Not Applicable; pathogen was not treated with this antibiotic.

Figure 1. Results of trend determination from the years 2009-2013 from the Cochran-Armitage and Somers' D tests.

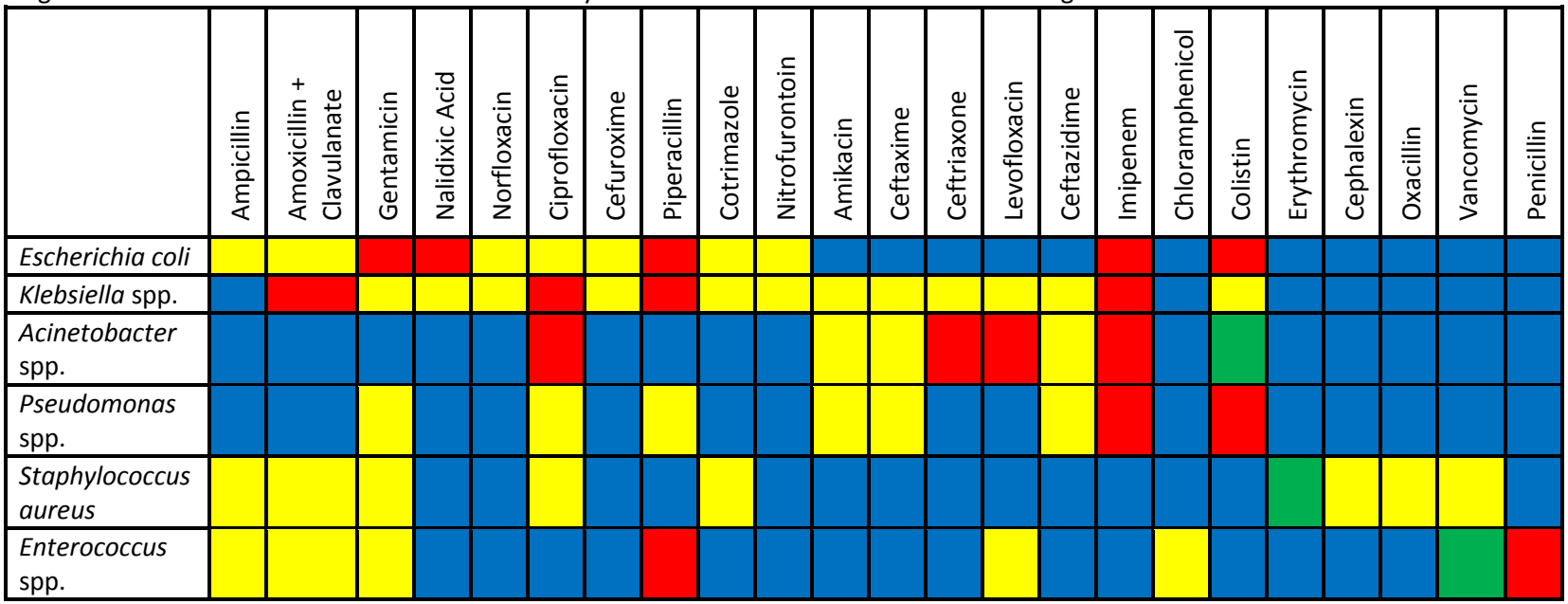


Figure 1. The above details the results of the Cochran-Armitage test and Somers' D test for time trend, examining the trend in bacterial susceptibility to specific antibiotics across the time period 2009-2013.

Key	
No trend	
Negative trend	
Positive trend	
Not tested	

Figure 2a. *E. coli* response to antibiotics with negative susceptibility trend over time.

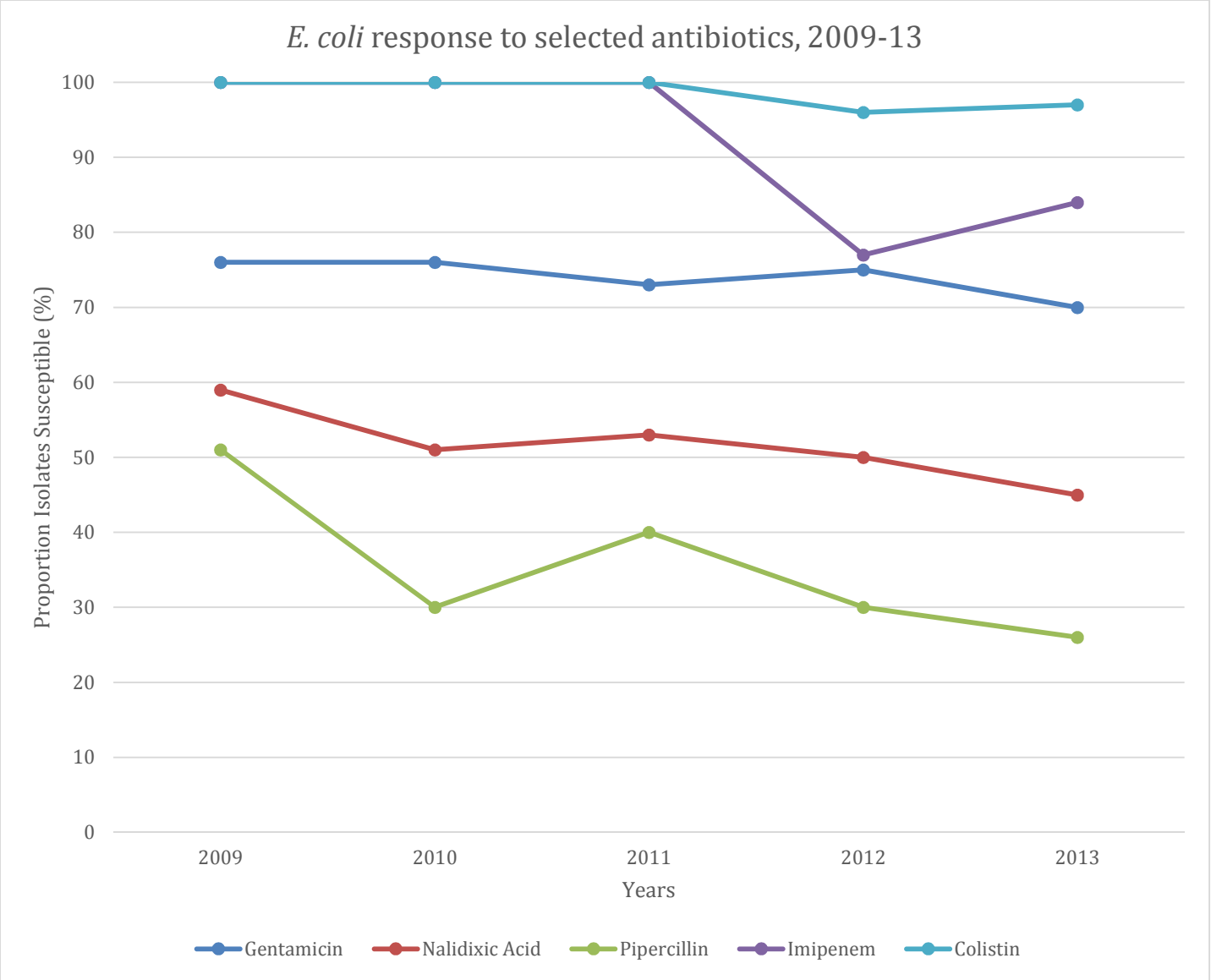


Figure 2b. *E. coli* response to all antibiotics, 2009-2013

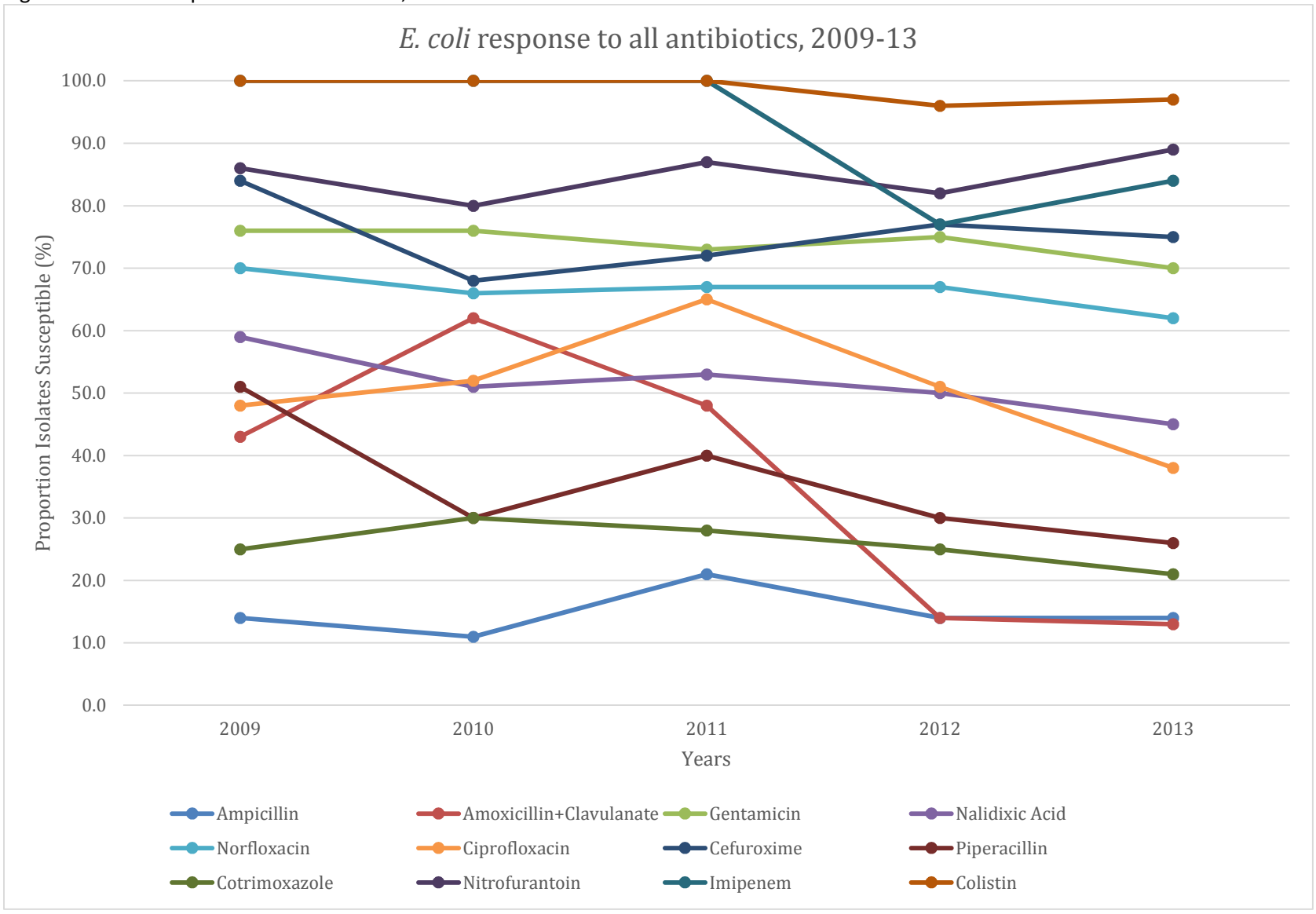


Figure 3a. *Klebsiella* spp. response to antibiotics with negative susceptibility trend over time.

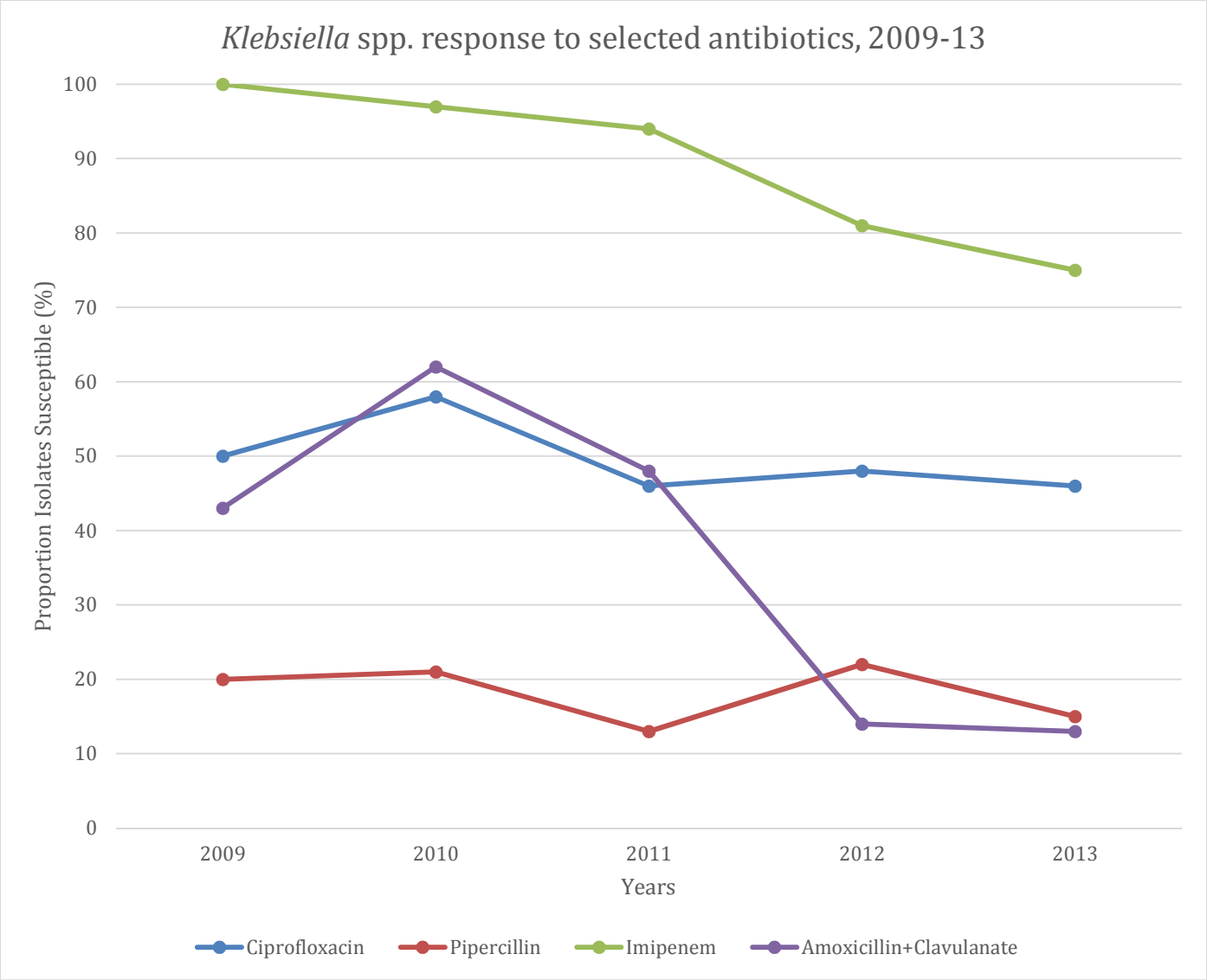


Figure 3b. *Klebsiella* spp. response to all antibiotics, 2009-2013

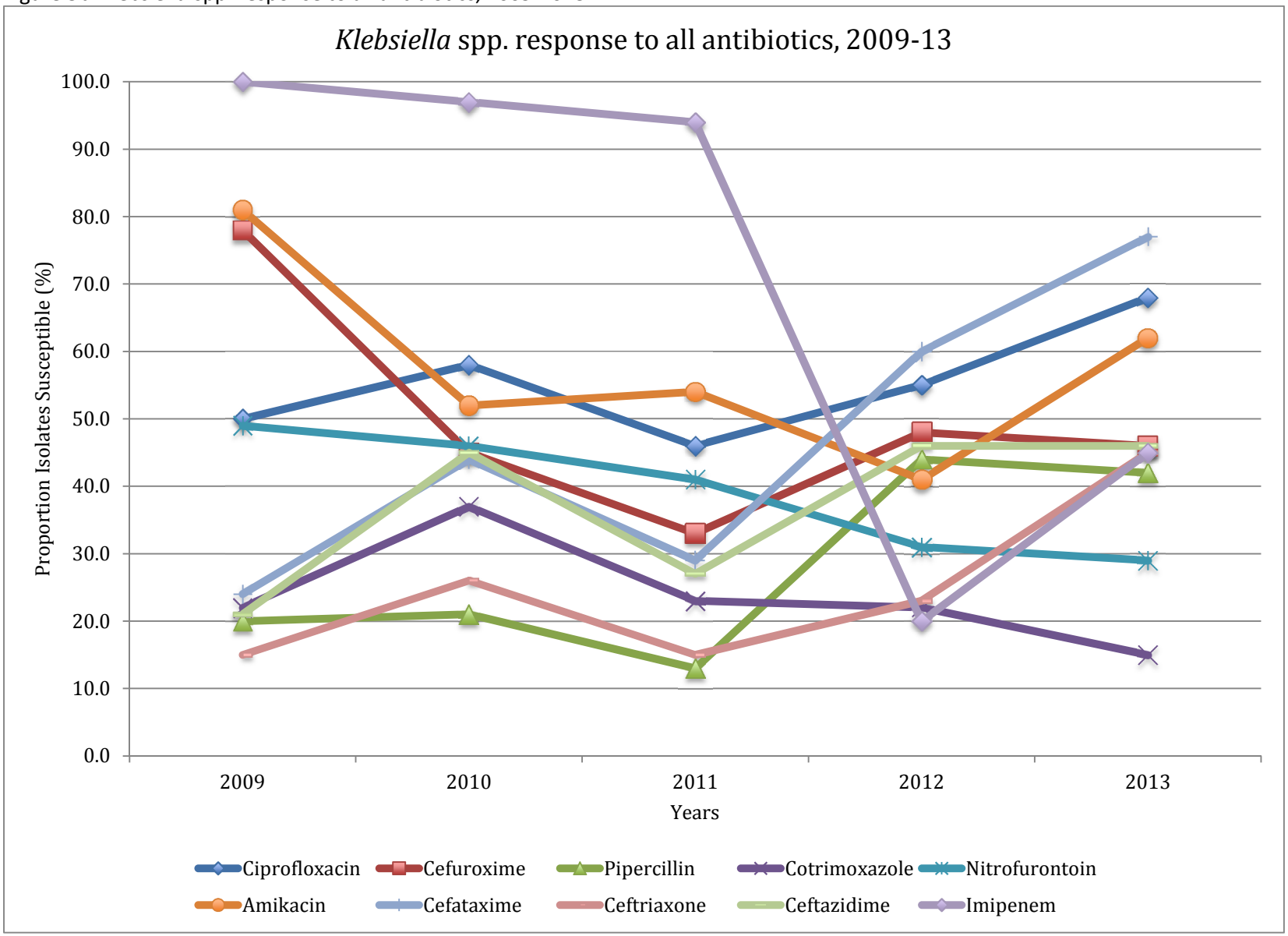


Figure 4a. *Acinetobacter* spp. response to antibiotics with negative susceptibility trend over time.

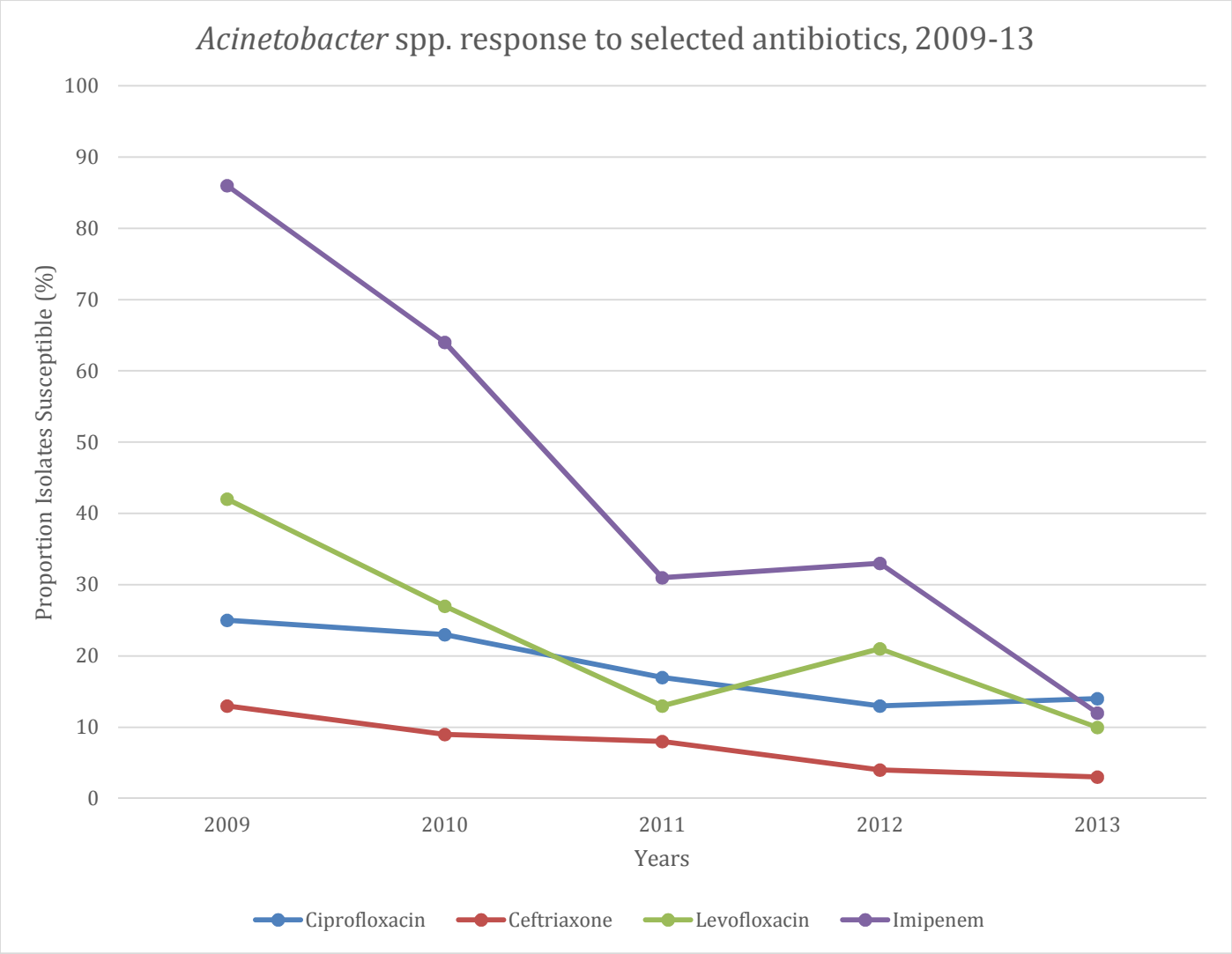


Figure 4b. *Acinetobacter* spp. response to all antibiotics, 2009-2013

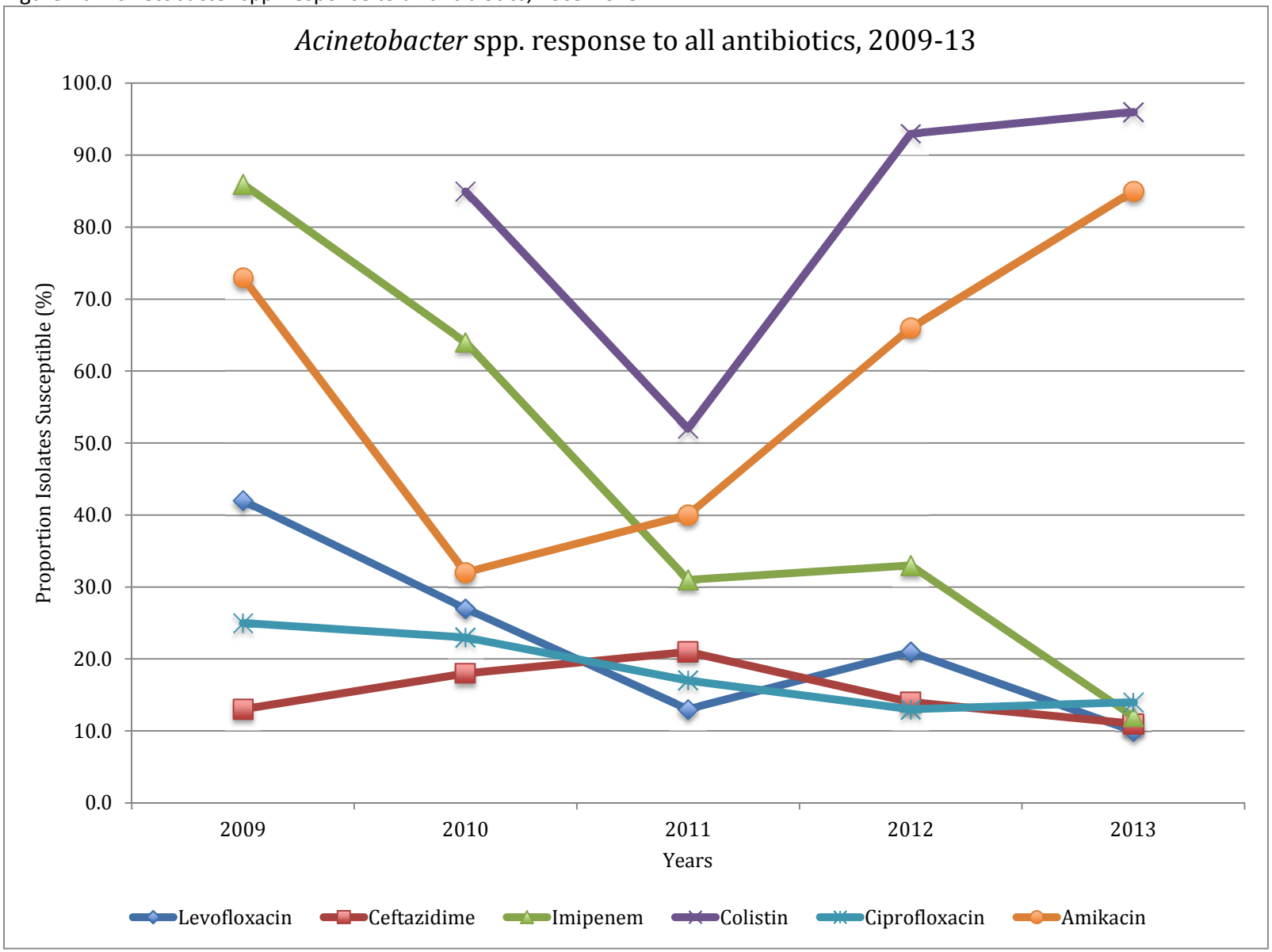
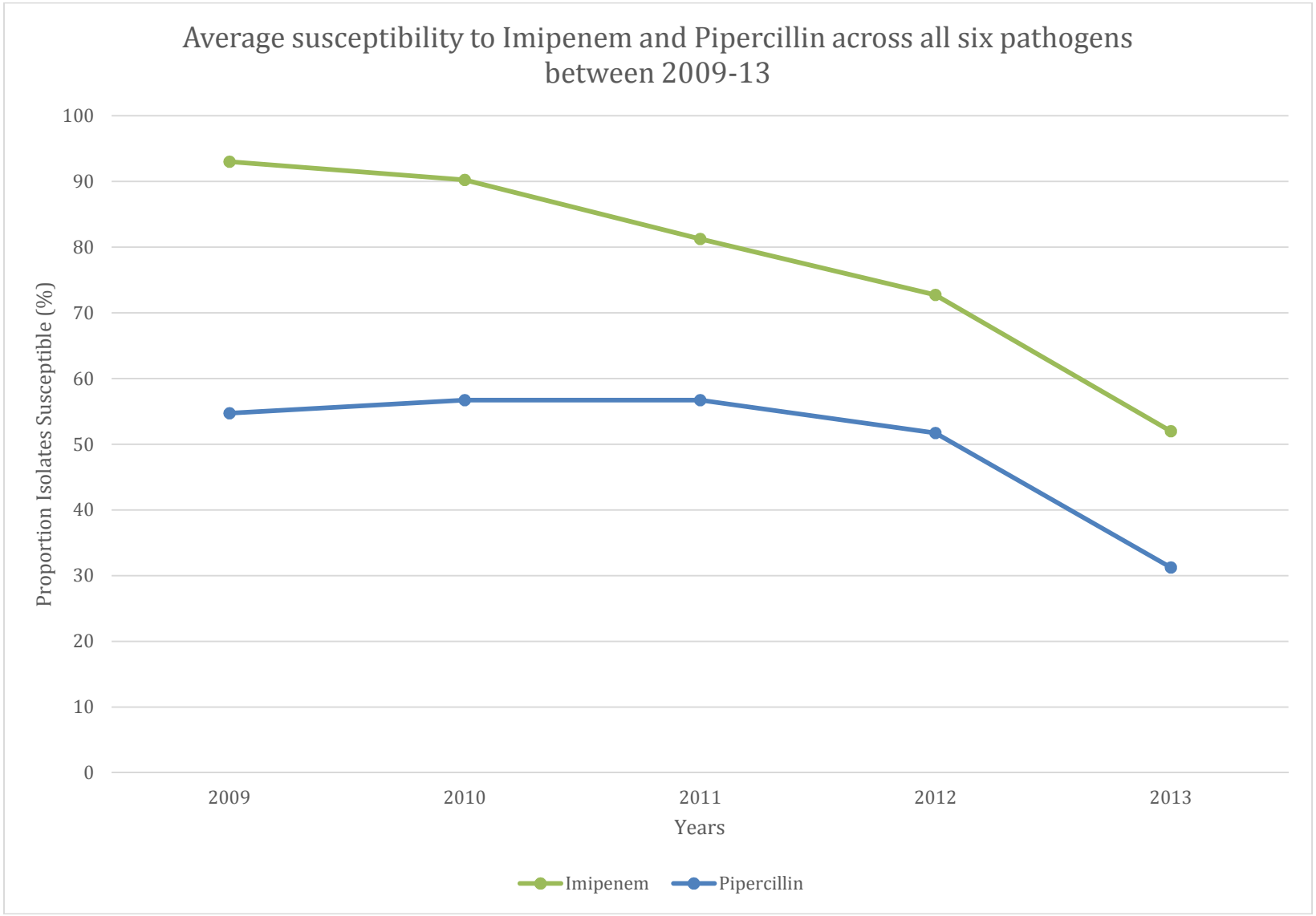


Figure 5. Imipenem and Piperacillin susceptibility. Graph shows the average susceptibility amongst all isolates per year during the period 2009-13.



Discussion:
Several studies have illustrated the growing threat of AMR in Sub-Saharan Africa, and there is a need for studies reporting on prevalence of resistant pathogens and the yearly trends of specific antimicrobial agents. This work sought to serve this need by examining the patterns of AMR for six unique bacterial pathogens, including *Escherichia coli*, *Klebsiella* species, *Acinetobacter* species, *Pseudomonas* species, *Staphylococcus aureus*, and *Enterobacter* species. These common bacterial isolates originated from clinical specimens of patients in the internal medicine wards of King Faisal Hospital in Kigali, Rwanda. Antibiotic sensitivity was recorded for a variety of pathogen-antibiotic groups during the five-year period from January 2009-December 2013.

Univariate statistical analysis revealed that the majority of bacterial isolates were *E. coli*, and the minority were *Acinetobacter* spp. This supports other recent studies from similar populations in Rwanda (3,8). Among all gram-negative isolates, Colistin exhibited the highest efficacy, although with high yearly variability in *Acinetobacter* spp. (81.5% average annual antibiotic sensitivity, with 20.21% standard deviation). As shown in Supplemental Tables 1a-d, sensitivity to Colistin remained relatively consistent throughout the time period. Imipenem was the second-most effective antibiotic amongst the gram-negative species, exhibiting antibiotic susceptibility proportions of 80% or greater in each of the species besides *Acinetobacter* spp., where is only yielded an average annual susceptibility proportion of 45.2%. Notably, *Acinetobacter* spp. consistently has the lowest average antibiotic sensitivity figures of all the gram-negative pathogens for each drug tested. This finding should be confirmed by further study. Among all gram-positive organisms, the antibiotic with the greatest overall effect was Vancomycin. It achieved 100% bacterial sensitivity for all five years for *S. aureus*, and a mean susceptibility of 99.4% (standard deviation, 1.34%) for *Enterococcus* spp. This is encouraging for the treatment of gram-positive species, as it suggests that there are still drugs with high sensitivity patterns, and potential for clinical longevity. Contrary to another recent study [3], we did not identify any Vancomycin resistance for *Staphylococcus aureus*. We also found that *S. aureus* was, on average, 97.8% sensitive to Oxacillin, or less than 3% resistant, in stark contrast to the finding that 82% of *S. aureus* strains were Oxacillin-resistant [3]. However, our results do corroborate the finding in the work of Nitrenganya et al. [3] that *E. coli* and *Klebsiella* isolates are at least 30% and 60% resistant, respectively, to a third-generation cephalosporin (Cefotaxime, Ceftriaxone). Similar to their findings, *E. coli* showed 8% resistance to Imipenem in the current study.

An analysis of antibiotic sensitivity over time indicated that the majority of bacterial groups yielded no trend. However, there were 17 negative trends identified, and 3 positive trends. The majority of negative susceptibility trends over time were identified in *E. coli*, *Klebsiella* spp., and *Acinetobacter* spp. Interestingly, Imipenem was the antibiotic that exhibited negative temporal trends for all three of these species, and it was found to have decreasing effectiveness over time for four species.
Similarly, Piperacillin showed a negative trend for three bacterial species, including *E. coli*, *Klebsiella* spp., and *Enterococcus* spp. (Figure 3).

This study had several limitations. Importantly, the dataset itself did not include susceptibility testing for all bacteria against all antiobiotics potentially used for treatment. Statistical variation was lost because of this limitation and because the results of various antibiotic susceptibility tests throughout a given year were collapsed into a single summary score. Future studies should attempt to capture a complete record of the multiple individual tests for each antibiotic-pathogen combination. Such data would provide more precise and nuanced information to inform clinicians and public health professions on important trends on the profile bacterial species' sensitivity responses to each antibiotic. Additionally, as only one data point was provided per year, trend analyses of only five data points per group precludes

drawing conclusive interpretations of drug sensitivity trends and our ability to inform policy recommendations.

Conclusions:

This study described trends of AMR in the King Faisal Hospital in Kigali, Rwanda. However, limitations to obtaining complete data regarding temporal trends of AMR in Rwanda, limited conclusive interpretations of our results. It is imperative that regular, consistent antibiotic sensitivity test be conducted on the common clinical isolates of patients. More complete data over a longer timeframe will provide more robust estimates of AMR and alert us to emerging resistant strains of bacteria. Further research might focus on the future of Imipenem and Piperacillin amongst gram-negative isolates in this clinical setting.

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APPENDIX

Information about drug classes and mechanism of action

(1) **ß-Lactam Antibiotics**: Penicillins, Cephalosporins, and Carbapenems

Mechanism of action: Beta-lactam antibiotics affect the biosynthesis of the murein layer of the bacterial cell wall. Traditionally these antibiotics have been effective because no analogous structure exists in mammalian cells, which means only bacteria are targeted, and host cells are protected [10]. The chemical structure of these antibiotics contains a ß-lactam ring. Certain side chains located on the ß-lactam ring allow the drugs to permeate the outer membrane of Gram-negative bacteria; once they have entered, they kill the organism[10].

Mechanism of Resistance: The modification of the drug by bacteria inactivates it. ß-lactamase enzymes hydrolyze the ß-lactam ring.

Examples used in this study:

- Ampicillin: Part of Aminopenicillin family; most effective against gram-positive species *S. pneumoniae*, *S. pyogenes*, *S. aureus*, and some *Enterococcus* spp.; gram-negative species *N. meningitidis*, *H. influenzae*, and some Enterobacteriacea.
- Amoxicillin: Aminopenicillin family; for use against acute otitis media, UTIs, Lyme Disease, *S. pneumoniae*.
- Cefalexin: First-generation Cephalosporin; for use against Gram-positive and some Gram-negative species.
- Cefotaxime: Third-generation Cephalosporin; for use against *S. aureus*, *S. pneumoniae*, *Klebsiella* spp., *Enterobacter* spp. and others
- Cefuroxime: Second-generation Cephalosporin; for use against Gram-positive bacteria
- Ceftriaxone: Third-generation Cephalosporin; broad-spectrum activity against Gram-positive bacteria and extended coverage of Gram-negative bacteria compared to second-generation drugs.
- Ceftazidime: Third-generation Cephalosporin; activity against Gram-positive and Gram-negative species.
- Imipenem: First-generation Carbapenem; given intravenously; broad-spectrum use for Gram-positive and Gram-negative pathogens; important for use against *Enterococcus* spp. and *P. aeruginosa*
- Oxacillin: Penicillin family; resistant to Penicillinases; for use against *S. aureus* and other Penicillinase-producing organisms
- Piperacillin: Ureidopenicillin family; given via intravenous or intramuscular injection; for use against *Pseudomonas* spp.

(2) **Anti-ribosomal Antibiotics**: Aminoglycosides and Macrolides

Mechanism of action: This second-largest class of antibiotics is effective because of structural differences in bacterial and eukaryotic ribosomes [10]. These drugs penetrate the outer membrane of Gram-negative organisms, then associate with a two-stage active transport system in the cell membrane. Finally, they bind the 30S ribosome subunit to inhibit protein synthesis and increase miscoding by the ribosomes, and ultimately increase the production of “nonsense” proteins that lead to the cell’s death [10].

Mechanism of Resistance: The modification of the drug by the bacterial R-plasmid-encoded enzyme, which results in reduced affinity for the ribosome and reduced transport into the cell [10].
Examples used in this study:

- Gentamicin: Aminoglycoside family; for use against Gram-negative species including *E. coli*
- Amikacin: Aminoglycoside family; used to treat multi-drug resistant Gram-negative bacteria like *Pseudomonas aerugrinosa*, *Acinetobacter* spp. and *Enterobacter* spp.
- Erythromycin: Macrolide family; typically prescribed for people with penicillin allergy; for use mainly against Gram-positive species, with limited Gram-negative use.

(3) **Anti-folate Antibiotics**: Sulfonamides
Mechanism of action: Capitalizes on the different ways eukaryotic cells and bacteria synthesize and use folic acid [10]. Humans require preformed folic acid, and are therefore unaffected by sulfonamides, which inhibit the synthesis of this compound. On the contrary, most bacteria that make folic acid lack a system for the uptake of preformed folic acid, so they die without it [10].
Mechanism of Resistance: Modification of the drug target, dihydropteroate synthase, makes the drug inactive.
Examples used in this study:

- Cotrimoxazole: Broad-spectrum drug effective against *Acinetobacter* spp., *E. coli*, *Klebsiella* spp., *S. aureus*, and many others (not to include *Enterococcus* spp. Or *Pseudomonas* spp.)

(4) **Quinolones and Fluoroquinolones**
Mechanism of action: This drug class works by inhibiting the action of bacterial topoisomerases—in Gram-positive bacteria, DNA gyrase, and in Gram-negative bacteria, topoisomerase IV. Bacteria are killed when topoisomerases are trapped while cutting DNA, which creates double-strand breaks in the bacterial chromosome [10].
Mechanism of Resistance: Mutations in the genes encoding DNA gyrase and topoisomerase IV cause reduced binding of the drugs.
Examples used in this study:

- Nalidixic Acid: Primarily for use against Gram-negative species like *E. coli*, *Enterobacter* spp., and *Klebsiella* spp.
- Norfloxacin: Second-generation Fluoroquinolone family; for use against bacteria causing urinary tract infections, including *E. coli*
- Ciprofloxacin: Second-generation Fluroquinolone; for use against Gram-negative species including *E. coli*, *Klebsiella* spp., *P. aeruginosa*, as well as Gram-positive species such as *Enterococcus faecalis*, *S. aureus*.
- Levofloxacin: Third-generation Fluroquinolone; for use against Gram-positive and Gram-negative species

(5) **Nitrofurantoin**:
Mechanism of action: This drug works by damaging bacterial DNA via nitrofuran reductase to create intermediates that attack ribosomal proteins, DNA, pyruvate metabolism, and other important cellular components [10]. For use against: *E.coli*, *Staphylococcus* spp., *Klebsiella* spp.
Mechanism of Resistance: Resistance may be chromosomal or plasmid-mediated, and involved inhibition of nitrofuran reductase.

(6) **Chloramphenicol:**
Mechanism of action: Slows growth of bacteria by inhibiting protein synthesis, via interference with protein chain elongation. It inhibits the peptidyl transferase activity of the bacterial ribosome [10]. For use against: Gram-positive and Gram-negative species, and most anaerobic species.
Mechanism of Resistance: Resistance to this drug occurs by three different ways—reduced membrane permeability; mutation of 50S ribosomal subunit, and elaboration of chloramphenicol acetyltransferase.

(7) **Colistin:**
Mechanism of action: This polymyxin antibiotic has both hydrophilic and lipophilic portions, which help to solubilize the bacteria cytoplasmic membranes, like a detergent [10].
For use against: highly-resistant Gram-negative infections.
Mechanism of Resistance: Resistance is rare, but has been described (cite this). Mechanism is unclear.

(8) **Vancomycin:**
Mechanism of action: This drug works by inhibiting proper cell wall formation in Gram-positive bacteria [10]. For use against *Enterococcus* spp., MRSA
Mechanism of Resistance: Resistance can be caused by either a change in the binding site in the peptidoglycan target, or restricted access to drug target.

Supplemental Table 1a. Antimicrobial susceptibilities of *Escherichia coli* isolates collected between 2009-2013

	No. of Isolates Tested				
Antimicrobial Agent	2009 (n= 415)	2010 (n= 429)	2011 (n= 469)	2012 (n= 579)	2013 (n= 581)
Ampicillin	14	11	21	14	14
Amoxicillin +Clavulanate	43	62	48	14	13
Gentamicin	76	76	73	75	70
Nalidixic Acid	59	51	53	50	45
Norfloxacin[a]	70	66	67	67	62
Ciprofloxacin	48	52	65	51	38
Cefuroxime	84	68	72	77	75
Pipercillin	51	30	40	30	26
Cotrimoxazole	25	30	28	25	21
Nitrofurontoin[a]	86	80	87	82	89
Amikacin	nt	nt	nt	nt	97
Cefataxime	nt	nt	nt	nt	80
Ceftriaxone	nt	nt	nt	nt	80
Levofloxacin	nt	nt	nt	nt	43
Ceftazidime	nt	nt	nt	nt	80
Imipenem	100	100	100	77	84
Chloramphenicol	nt	nt	nt	nt	57
Colistin	100	100	100	96	97

[a] for urine isolates only

nt: The pathogen was not tested with this antibiotic at this time point.

Supplemental Table 1b. Antimicrobial susceptibilities of *Klebsiella* spp. isolates collected between 2009-2013

	No. of Isolates Tested				
Antimicrobial Agent	2009 (n= 138)	2010 (n= 193)	2011 (n= 219)	2012 (n= 235)	2013 (n= 190)
Amoxicillin +Clavulanate	31	44	26	7	15
Gentamicin	56	55	48	48	52
Nalidixic Acid	62	70	46	40	55
Norfloxacin[a]	89	79	58	55	68
Ciprofloxacin	50	58	46	48	46
Cefuroxime	78	45	33	44	42
Pipercillin	20	21	13	22	15
Cotrimoxazole	22	37	23	31	29
Nitrofurontoin[a]	49	46	41	41	62
Amikacin	81	52	54	60	77
Cefataxime	24	44	29	23	25
Ceftriaxone	15	26	15	11	57
Levofloxacin	nt	nt	46	46	46
Ceftazidime	21	45	27	20	45
Imipenem	100	97	94	81	75
Chloramphenicol	nt	nt	nt	nt	4
Colistin	100	100	100	96	100

[a] for urine isolates only

nt: The pathogen was not tested with this antibiotic at this time point.

Supplemental Table 1c. Antimicrobial susceptibilities of *Acinetobacter* spp. isolates collected between 2009-2013

	No. of Isolates Tested				
Antimicrobial Agent	2009 (n= 33)	2010 (n= 58)	2011 (n= 69)	2012 (n= 94)	2013 (n= 59)
Ciprofloxacin	25	23	17	13	14
Amikacin	73	32	40	66	85
Cefataxime	11	5	12	5	7
Ceftriaxone	13	9	8	4	3
Levofloxacin	42	27	13	21	10
Ceftazidime	13	18	21	14	11
Imipenem	86	64	31	33	12
Colistin	nt	85	52	93	96

[a] for urine isolates only
nt: The pathogen was not tested with this antibiotic at this time point.

Supplemental Table 1d. Antimicrobial susceptibilities of *Pseudomonas* spp. isolates collected between 2009-2013

	No. of Isolates Tested				
Antimicrobial Agent	2009 (n= 25)	2010 (n= 52)	2011 (n= 94)	2012 (n= 118)	2013 (n= 86)
Gentamicin	65	78	91	77	74
Ciprofloxacin	80	86	89	81	78
Pipercillin	71	81	83	81	69
Amikacin	88	51	93	74	78
Cefataxime	82	35	56	32	42
Ceftazidime	65	93	89	84	74
Imipenem	nt	100	100	100	37
Colistin	nt	100	100	100	88

[a] for urine isolates only
nt: The pathogen was not tested with this antibiotic at this time point.

Supplemental Table 1e. Antimicrobial susceptibilities of *Staphyloccocus aureus* isolates collected between 2009-2013

	No. of Isolates Tested				
Antimicrobial Agent	2009 (n=118)	2010 (n= 140)	2011 (n= 102)	2012 (n= 135)	2013 (n= 122)
Ampicillin	12	20	29	22	17
Amoxicillin +Clavulanate	51	20	76	30	26
Gentamicin	79	82	94	89	92
Ciprofloxacin	86	83	71	98	87
Cotrimoxazole	67	76	65	97	55
Erythromycin	47	71	73	69	60
Cephalexin	79	88	88	83	94
Oxacillin	96	98	98	99	98
Vancomycin	100	100	100	100	100

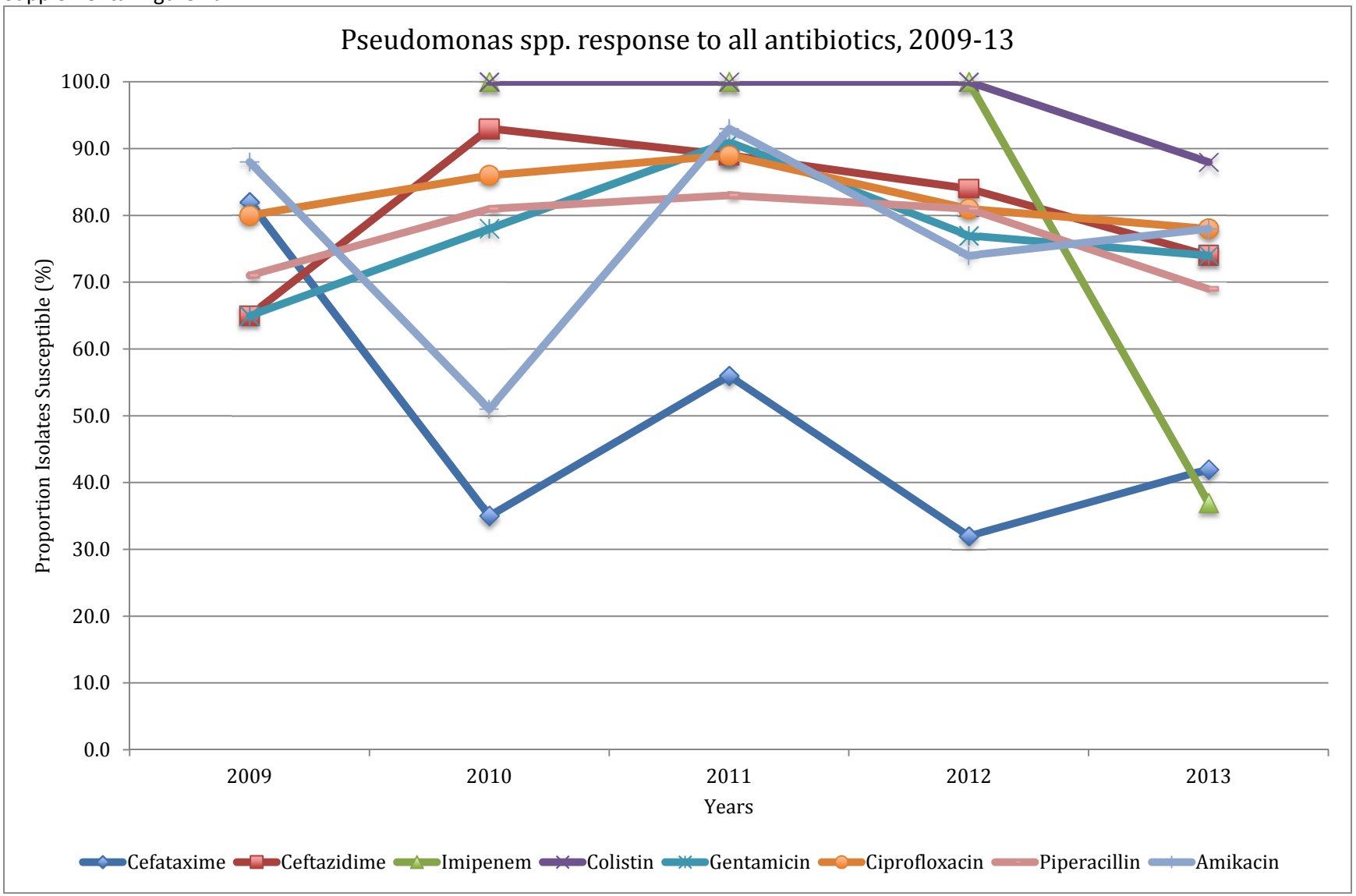
[a] for urine isolates only
nt: The pathogen was not tested with this antibiotic at this time point.

Supplemental Table 1f. Antimicrobial susceptibilities of *Enterococcus* spp. isolates collected between 2009-2013

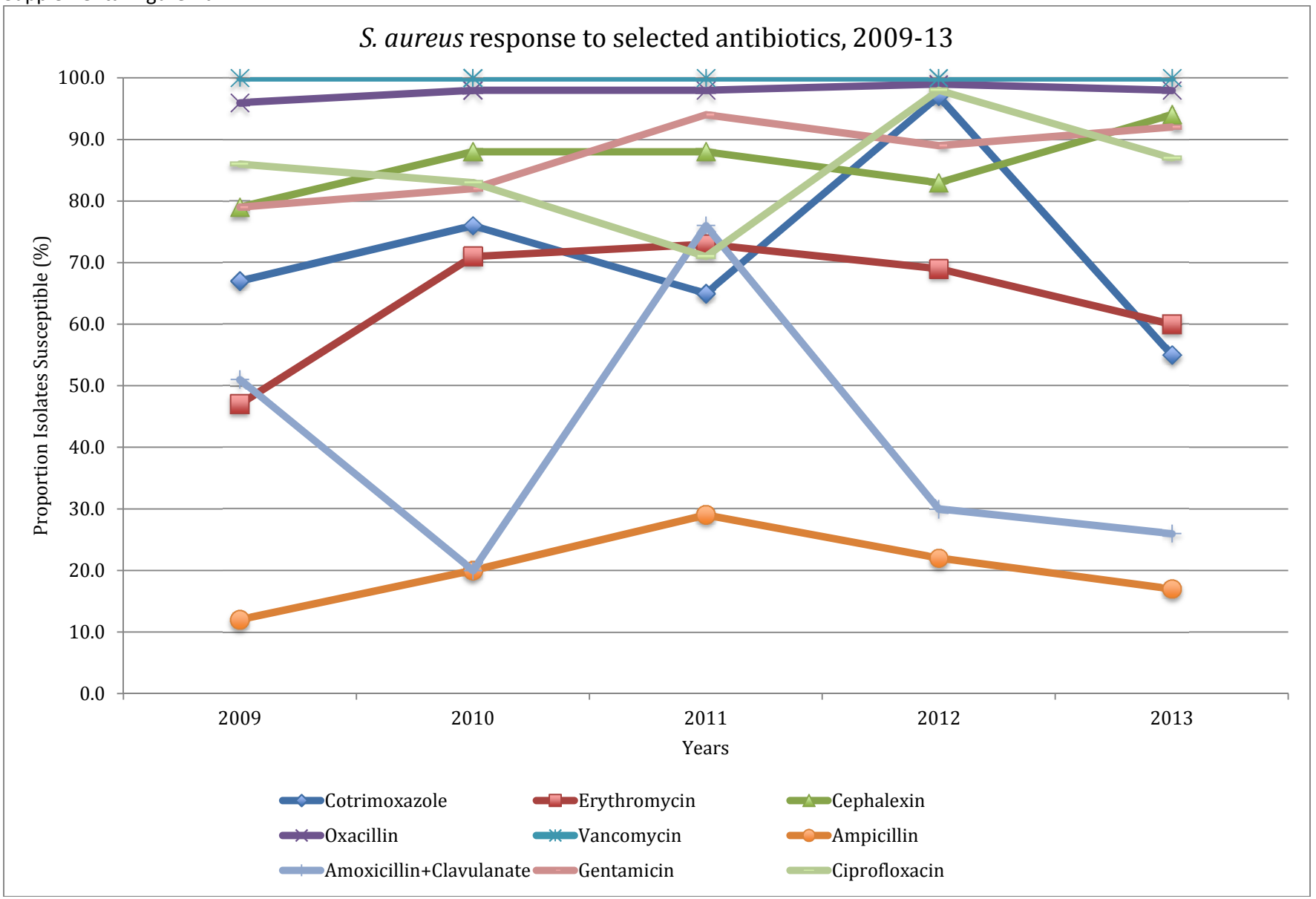
	No. of Isolates Tested				
Antimicrobial Agent	2009 (n= 56)	2010 (n= 113)	2011 (n= 85)	2012 (n= 157)	2013 (n= 132)
Ampicillin	80	85	82	83	83
Amoxicillin +Clavulanate	80	96	91	86	92
Gentamicin	24	27	26	21	37
Pipercillin	77	95	91	74	15
Levofloxacin	29	64	73	65	42
Chloramphenicol	65	46	69	51	68
Vancomycin	97	100	100	100	100
Penicillin	nt	45	26	17	19

[a] for urine isolates only
nt: The pathogen was not tested with this antibiotic at this time point.

Supplemental Figure 1a.



Supplemental Figure 1b.



Supplemental Figure 1c.