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Epidemiology and Antimicrobial Resistance of Clinical *Escherichia coli* Isolates from Selected Hospitals in Saudi Arabia

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Abstract

Multidrug-resistant (MDR) of Escherichia coli (E.coli) has been an important public health problem in Saudi Arabia, causing therapeutic failure with a correspondingly high health burden. To validate the prevalence and susceptibility of patient-isolated MDR E.coli in separate hospitals in Makkah, Saudi Arabia. Period in between from May to Nov 2016 were five distinct bacterial species isolated from the culture of urine. E. coli (n=77), Proteus mirabis (n=10), Klebsiella pneumoniae (n=38), Klebsiella aerogenes (n=10) and Enterobacter cloacae (n=11), but the most commonly detected organisms were Escherichia coli (n=77) isolated from clinically distinct specimens, classified, tested for susceptibility to antimicrobials and screened for Extended Spectrum Beta-Lactamase (ESBL) developed utilizing as per normal methods. E. coli (n=77) isolates from patients, the bulk of which were from urine (41%). Of these, resistance rates to Amikacin (AMK) have been identified (16/146); 10.95% (p<0.36), Amox/K Calv (AMC); (34/146) 23.28% (p<0.70), Ampicillin (AMP); (146/146) 100% (p<1), Cefazolin (CFZ); (145/146) 99.31% (p<0.97), Cefepime (FEP); (146/146) 100% (p<1), Cefotaxime (CTX); (5/146) 3.42% (p<0.19), Cefotaxime/ K Calvulanta (CT/CTL) (0/146) 0%, Cefoxitin (FOX); (25/146) 17.12% (p<0.63), Ceftazidime (CAZ) (4/146) 2.73 % (p<0.25), and Ceftazidime/ K Calvulantae (TZ/TZL); (0/146) 0%. Overall, 50% MDR of E. coli was resistant to >4 antimicrobial agents and 279 (7%) of ESBL were detected. Female isolates (59%) were more resistant than male isolates (41%), (p<0.05). Drug-resistance monitoring and clinical data epidemiological review require additional periodic information on appropriate antimicrobial resistance management.

Keywords: *Multi-Drug Resistance; E. coli; esbl; Minimal Inhibitory Concentration*

Introduction

Antimicrobial Resistance (AMR) has developed as one of the top ten significant human health challenges in the last ten years. One of the critical threats to human health is considered [1]. Health care services may not be successfully treated resistant bacterial infections in

the next twenty years [2]. Bacterial antibiotic-resistant diseases cause patients to spend more time in hospitals, resulting in higher treatment expenses [3]. Antibiotic-resistant infectious agents are responsible for nearly ~700,000 deaths every year, with an estimated ~10 million peoples suffering from cancer every year [4] Indeed, even at existing rates, it is right to assume that more than one million people would have died from AMR. However, microorganisms' ability to mutate so that the medicine no longer works when exposed to antimicrobials has led to treatments' inefficiency. The excessively over and misuse of antibiotics intensifies the development and dissemination of drug-resistant bacteria [5]. Even if people should not alter the way antibiotics are being used today, these new antibiotics will suffer the same fate as the existing ones and become inactive.

Saudi Arabia has been well known as a country with a growing AMR, a challenge for the Kingdom's health authorities. Based on these findings, non-prescription over-the-counter antibiotics in the Saudi community pharmacies contribute to the inappropriate use of antibiotics. Just one out of ~88 pharmacists in the eastern province declined to market antibiotics without a prescription, and 77.6% of Riyadh pharmacies prescribed non-prescription antibiotics [6].

Therefore, during the Hajj (pilgrimage) period, it is attributed to the vast population of expatriates holy city Makkah pilgrims. Recent studies have shown that returning travelers from Hajj have acquired MDR. Acinetobacter baumannii and New Delhi Metallo β-Lactamase (NDM) producing *E.coli* during the Hajj event. Previous results from two leading hospitals in Makkah show that 24.6% of *E. coli* was ceftazidime-resistant antibiotics, 34.4% of *Klebsiella pneumoniae*, and 52.7% of Pseudomonas aeruginosa [7]. No data reveals the lack of a national monitoring program for AMR and healthcare-acquired infections in Saudi Arabia. No studies have been performed in various clinics, Makkah to determine people's knowledge and understanding of antibiotic resistance. The findings of this analysis would also include details on AMR awareness in Makkah. This research would also help prepare the public care division and implement new education techniques and initiatives to encourage the effective use of antibiotics in the general population to reduce AMR's rise in KSA. Therefore, this study was intended to ascertain AMR awareness in the general population of the Makkah Region.

The research goals were to retrieve raw data from the medical laboratory information system and then enter it into a unique dataframe that requires knowledge of statistical programming. In this analysis, open-source and commercial applications are used to visualize the prevalence of antibiotic resistance profiling in routine clinical samples in Saudi Arabia based on raw data from clinical sampling. Bacterial isolates are screened for antibiotic resistance using a variety of techniques. Therefore, antimicrobial sensitivity was expressed either as a Minimum Inhibitory Concentration (MIC) compared to other antibiotics, estimated through dilution method or an E-test, often as an inhibition zone diameter when assessed using a disk diffusion system that can be used to determine dendrograms and perform various statistical analyses. Finally, to confirm the antibiotic is most appropriate based on MIC and to proceed with the other most relevant things to confirm microorganism's resistance.

Materials and Methods

Sample collection

The samples (Umbilical, Urine, HVS, PUS, TRA, Wound, Nasal swab, Groin swab, High Vaginal swab, Vagina, and Cervical) were obtained from three separate hospitals throughout the Makkah area during the last six months of May to November 2016 for E. coli isolation (Figure 1). Unique susceptibility tests using commercial systems might include bacterial colonies from culture were used to detect resistance to specific antimicrobial drugs by using molecular techniques.

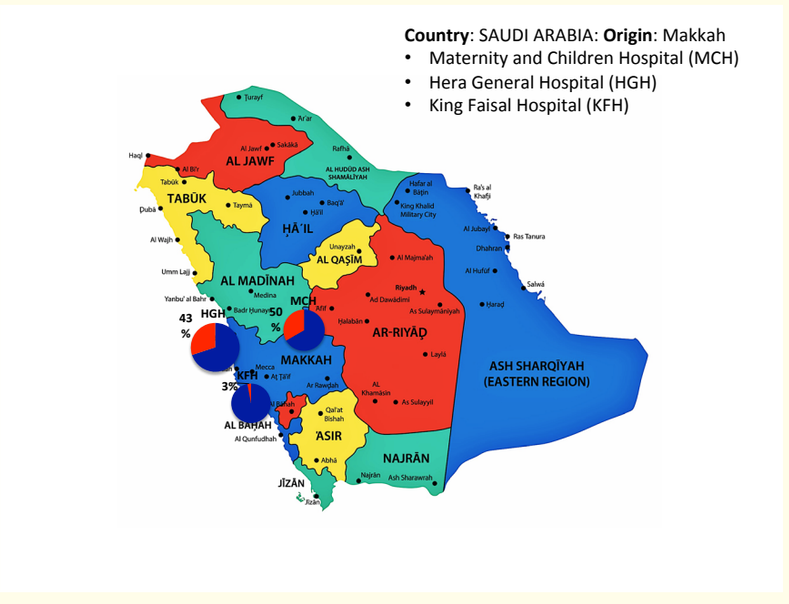

Figure 1: *Model accuracy for the patients isolated from different sources.*

Sample size estimation for study design

A statistical approach was applied to measure the number of isolates of clinical samples required for biostatistics screening (Daniel WW, 1999). The sample size depended on the precision required for estimating the prevalence of resistance observed over a given duration. Therefore, the sample size depends on the original or predicted occurrence of the resistance and the number of populations to be observed, and the appropriate degree of statistical significance and potential to distinguish the difference.

The sample size (n) is determined based on the formula

$$n = \frac{z2 \cdot p \cdot \frac{1-p}{e}2}{1+(z2 \cdot p \cdot \frac{1-p}{e^2 \cdot N})}$$

Whereas

n: Size of the sample,

z: Z-score correlated with the level of confidence,

p: Proportion of the sample, expressed as decimal,

e: Margin of error, expressed as a decimal,

N: Scale of population.

Such that: $z = 1.96$ for a significant at 95% confidence rating, p = proportion (expressed as decimal), N = size of population, *e* = margin of sampling error.

$$z = 1.95, p = 0.5, N = 235, e = 0.05$$

$$n = \frac{1.962 \cdot 0.5 \cdot \frac{1-0.5}{0.052}}{1+(1.962 \cdot 0.5 \cdot \frac{1-0.5}{0.052 \cdot 235})}$$

$$n = 384.16 \ / \ 2.6347 = 145.807$$

$$n \approx 146 \ patients$$

The study sample with a fixed population correction is ~146.

The study samples were obtained from the Maternity and Children's Hospital, King Faisal Hospital, and Hera General Hospital. Using a standard random sampling method, a barcode number was allocated to each patient, and the requested sample was selected at random. Samples were selected to reflect on the national population census of Saudi Arabia in terms of sex, age, place of residence, and population size. Explanatory variables information on antimicrobial resistance, including participants in the study, and independent variables of characteristics, antibiotic resistance factors over several types of diseases that can be treated with antibiotics and antibiotic resistance complications.

Isolation and Identification of bacterial pathogens

Samples were preserved back to the laboratory on ice within 6–10h of sampling time for isolation and characterization of the E. coli. cultures were inoculated on MacConkey agar media using sterile cotton swabs and 24 hours at 37°C in an alkaline medium. Five single red colonies of each sample were collected for the further purification of even the colony. The colonies were consequently classified using standard biochemical and API20 assays (bioMérieux, Durham, NC, USA). Both of them positively identified E. coli strains, and one strain per patient was preserved at −80°C in Luria-Bertani (LB) 30% glycerol-containing broth.

Statistical analysis and modeling

Data interpretation was calculated using SAS/STAT version 12.1, North Carolina State University, U.S.A. Empirical factors are presented based on the standard deviation (S.D.). Qualitative factors are shown using frequency distribution tables and percentages. A model mixed Analysis of Variance (ANOVA) (Rouder et al., 2016) methods for examining clustered data have been widely used to analyze data on antimicrobial resistance, despite the potential use of these techniques. Besides, the contrast of multiple treatments was calculated by one-way ANOVA with post-hoc Tukey HSD (Genuinely Significant Difference). One-way ANOVA with post-hoc Tukey HSD analysis integrated with Scheffé, Bonferroni as well as Holm multiple comparisons for antimicrobial resistance analysis. Tukey HSD was used for Tukey-Kramer, where treatment within the study groups had unequal findings in imbalanced observations. Every fair value was found to be significant at $p>0.05$. Other than modeling techniques, such as logistic regression analyzes, are reported for the categorical study of antimicrobial resistance.

Ethical consideration

The research protocol has been reviewed and approved by the local ethics commissions. All procedures performed in this research studies were in accordance with ethical standards of the institutional and national research board. The approval letter was issued from IRB-Makkah with the reference number (H-02-K-076-0320-281).

Results

Sample collection and validation

The occurrence of different pathogenic microorganisms collected from variable diagnostic specimens (n=146) gathered from various hospitals (King Faisal Hospital: KFH, Hera General Hospital: HGH and Maternity and Children Hospital: MCH) in Makkah state was shown in Figure 2. Urine (n=41), umbilical (n=2), cervical (n=6), vagina (n=1), high vaginal swab (n=6), groin swab (n=12), nasal swab (n=6) wound (n=10), TRA (n=3), PUS (n=7), HUS (n=9) and other specimens (n=42) (Figure 3) constitute a large portion of the specimens. The samples were isolated from across all age groups: (n=90) female and (n=56) male. Of the maximum population of isolates (n=146), adult patients were identified. Five different pathogenic bacterial species were isolated from the urine culture (E. coli n=77, Enterobacter cloacae (n=11), Proteus mirabilis n=10, Klebsiella pneumoniae n=38, Klebsiella aerogenes n=10), but perhaps the most commonly identified species were Escherichia coli (n=77). The frequency of pathogenic microorganisms was significant statistically and differing between males and females ($p<0.0001$). Extended-spectrum beta-lactamases were identified in the period collected (n=279) between May to November 2016. Of these, n=279 was the overall isolated percentage 77 were Escherichia coli (50%) of the total for each species, 38 was Klebsiella pneumoniae (50%), 10 was Proteus mirabilis (50%), 11 was Enterobacter cloacae (50%) and 10 remained Klebsiella aerogenes (50%).

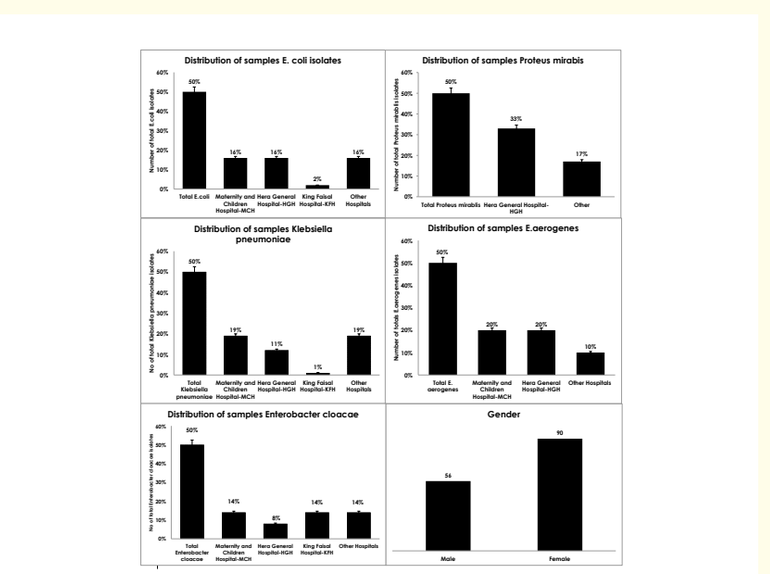

Figure 2: *Distribution of E. coli isolates within the different hospitals MCH, HGH, KFH and genders.*

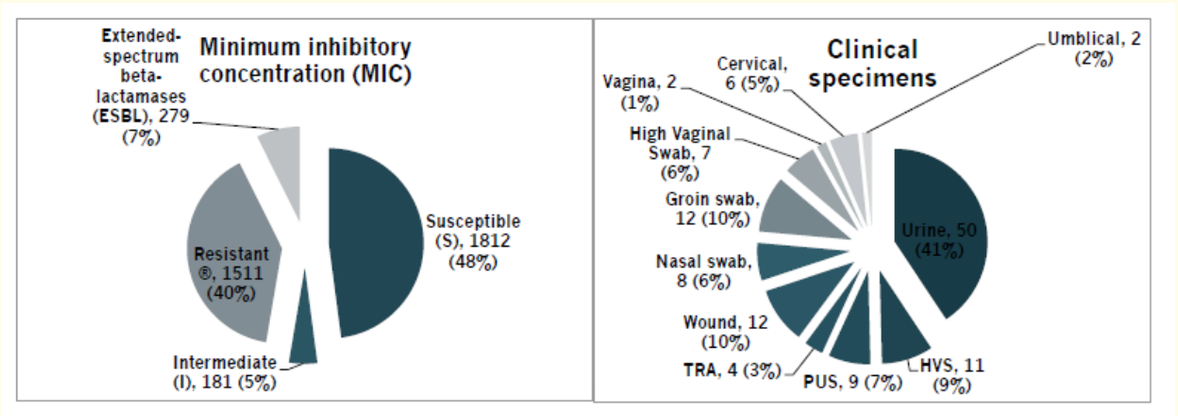

Figure 3: *Model accuracy for the patients isolated from various sources and total of minimum inhibitory concentration.*

Antibiotic resistance profile and related bacterial strains

The antibiotic-resistant bacteria characteristic among all standardized ~40 strains of bacteria was assessed using their susceptibility or resistance assays by cultivating the bacteria individually upon this LBA substrate containing various antibiotics. The resistant bacteria profile of all distilled ~41 bacterial strains was calculated susceptibility or sensitivity assays by promoting the bacteria separately on the LBA medium different specific antibiotics. Of the ~41 bacterial strains tested, 45% were resistant to Ampicillin (100%) and Cefepime (100%) without leaving these antibiotic-sensitive bacteria tested. Other than the bacterial strains tested, Cefazolin (i.e., 99%) was resistant, leaving fewer than 1% of the bacteria tested resistant to these antibiotics. The rest of the bacterial resistance was sensitive to (Amikacin 123/146: 88%), Amox/ K Calv (74/146: 50%), and Cefoxitin (120/146: 82%). Which indicates that these are the most efficient antibiotics on these bacterial strains tested (Figure 4 and 5), whereas the other six tested antibiotics had a marginal effect (roughly 45% Intermediate) on the sewage reveals that bacterial strains bacteria. Antibiotics' impact as bacterial growth-inhibiting variables has led the clustering into gentle and distinguished treatment within groups. The most detailed cluster-based study was conducted in groups containing bacterial strains most resistant to Ampicillin, Cefepime, and Cefazolin. The other clusters indicated differing antibiotic resistance potential of different bacterial strains studied. E.g., cluster-2 showed resistance to β-lactam antibiotics, consisting of bacterial strains, showing resistance to Cefotaxime (141/146: 96% and Ceftazidime (140/146: 95%).

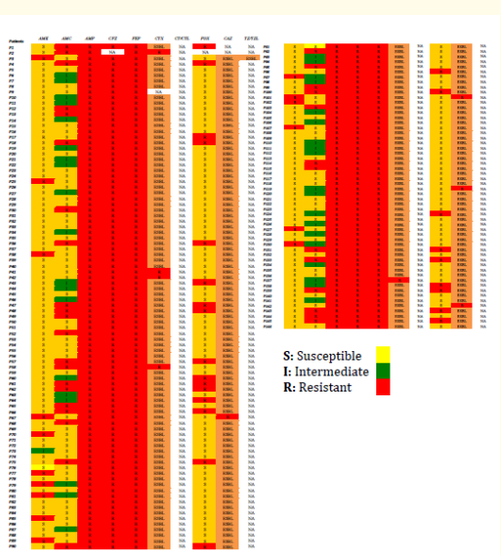

Figure 4: *Antibiotic resistance profiling of isolated bacterial strains by Cluster analysis. Isolates are typically clustered based on their resistance categories using a categorical coefficient based on different states' values. The corresponding colors of each antibiotic category, i.e., S: susceptible (green), I: intermediate (orange), and R: resistant (red).*

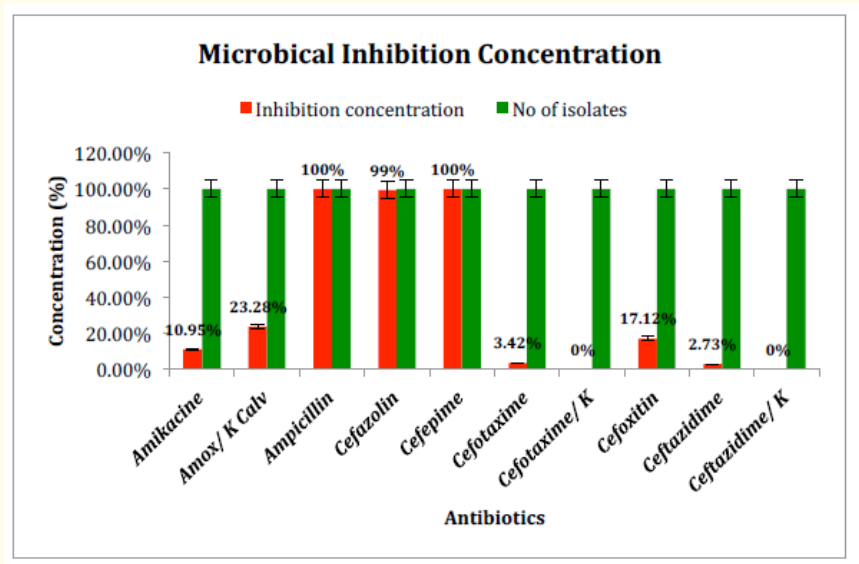

Figure 5: *Microbical inhibition concentration and of isolated bacterial strains of the tested antibiotics between the studied ESBL-positive E. coli isolates. The Y-axis values are expressed in percentage.*

Statistically Significant difference of antibiotics

Holm (1979) [10] suggested a significant development in the Bonferroni process. Among the numerous evaluations of the Holm method's merits and its standardized advantages over the Bonferroni method [11] were prominent. The antimicrobial resistance data (Table 1) was analyzed based on these algorithms' sources to compare the Holm method. All the statistical programs that were integrated the Holm method. The significance of the p-value correlating here to F-statistic from one-way ANOVA was less than p=≤0.05, indicating that the antibiotic resistance over one and most different bacterial species to be treated were substantially different (Table 2,3). After one-way ANOVA in SPSS for multiple comparative checks of Scheffé, Bonferroni, and Holm multiple, we used turkey HSD. These post-hoc experiments established pairs forms of treatment are substantially different from each other.

Treatment →	Amikacin	Amox/ K Calv	Ampicillin	Cefazolin	Cefepime	Cefotaxime	Cefotaxime/ K Calvulanta	Cefoxitin	Ceftazidime	Ceftazidime/K Calvulantae
Minimum Inhibitory Concentration (MIC)										
Patient1	<=16	>=16/8	>=16	>=16	>=16	>=32	<=05	>=8	0	0
Patient2	0	0	0	0	0	0	0	0	0	0
Patient3	>=32	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	>=16
Patient4	<=16	>=16/8	>=16	>=16	<=8	32	4	>=8	>=16	>2
Patient5	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient6	<=16	16/8	>=16	>=16	16	>=32	<=05	<=8	16	<=025
Patient7	<=16	16/8	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient8	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient9	<=16	<=8/4	<=8	<=8	<=2	<=05	<=05	<=8	16	<=025
Patient10	<=16	16/8	>=16	>=16	>=16	>=32	<=025	<=8	>=16	<=025
Patient11	<=16	16/8	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient12	<=16	>=16/8	>=16	>=16	>=16	>=32	4	<=8	>=16	>2
Patient13	<=16	>=16/8	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient14	<=16	16/8	>=16	>=16	>=16	>=32	4	<=8	>=16	>2
Patient15	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	16	<=025
Patient16	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	8	<=025
Patient17	>=32	<=8/4	>=16	>=16	<=8	16	<=05	>=8	4	<=025
Patient18	<=16	>=16/8	>=16	>=16	>=16	>=32	4	>=8	16	>2
Patient19	<=16	16/8	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient20	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	8	<=025
Patient21	<=16	16/8	>=16	>=16	>=16	>=32	<=05	<=8	16	<=025
Patient22	<=16	16/8	>=16	>=16	>=16	>=32	<=025	<=8	>=16	<=025
Patient23	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	8	<=025
Patient24	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient25	>=32	<=8/4	>=16	>=16	>=16	>=32	4	<=8	>=16	>2
Patient26	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	8	<=025
Patient27	<=16	16/8	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient28	<=16	<=8/4	<=8	<=8	<=8	>=32	<=05	<=8	4	>2
Patient29	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient30	<=16	>=16/8	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient31	<=16	<=8/4	>=16	>=16	>=16	>=32	>4	<=8	>=16	<=025
Patient32	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	16	<=025
Patient33	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	8	<=025
Patient34	<=16	16/8	>=16	>=16	>=16	>=32	<=05	<=8	16	<=025
Patient35	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	16	<=025

Patient36	<=16	>=16/8	>=16	>=16	>=16	>=32	<=05	>=8	16	<=025
Patient37	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient38	>=32	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient39	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	8	<=025
Patient40	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	4	<=025
Patient41	<=16	<=8/4	>=16	<=8	<=8	<=2	<=05	<=8	8	<=025
Patient42	<=16	<=8/4	>=16	<=8	<=8	<=2	<=05	<=8	8	<=025
Patient43	<=16	16/8	>=16	>=16	>=16	>=32	<=05	>=8	>=16	<=025
Patient44	<=16	16/8	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient45	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient46	<=16	16/8	>=16	>=16	<=8	>=32	<=05	<=8	4	<=025
Patient47	<=16	>=16/8	>=16	>=16	<=8	32	4	>=8	8	2
Patient48	<=16	>=16/8	>=16	>=16	>=16	>=32	4	>=8	8	2
Patient49	<=16	>=16/8	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient50	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	16	<=025
Patient51	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient52	<=16	>=16/8	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient53	<=16	<=8/4	>=16	>=16	>=16	>=32	4	<=8	>=16	<=025
Patient54	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	16	<=025
Patient55	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	16	<=025
Patient56	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	16	<=025
Patient57	<=16	>=16/8	>=16	>=16	>=16	>=32	<=05	>=8	16	2
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Patient59	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient60	<=16	16/8	>=16	>=16	>=16	>=32	<=05	>=8	16	<=025
Patient61	<=16	>=16/8	>=16	>=16	>=16	>=32	4	>=8	>=16	2
Patient62	<=16	>=16/8	>=16	>=16	16	>=32	4	>=8	>=16	>2
Patient63	<=16	16/8	>=16	>=16	>=16	>=32	<=05	<=8	4	<=025
Patient64	<=16	16/8	>=16	>=16	>=16	>=32	<=05	>=8	16	<=025
Patient65	<=16	>=16/8	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient66	<=16	>=16/8	>=16	>=16	>=16	>=32	4	>=8	>=16	>2
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Patient69	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient70	>=32	<=8/4	16	>=16	<=8	>=32	<=05	<=8	8	>2
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Patient72	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient73	32	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient74	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient75	<=16	>=16/8	>=16	>=16	>=16	>=32	4	>=8	>=16	(>2)
Patient76	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient77	>=32	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient78	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	4	<=025
Patient79	>=32	16/8	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient80	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient81	>=32	16/8	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient82	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient83	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	8	<=025
Patient84	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient85	>=32	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient86	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient87	<=16	16/8	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient88	<=16	<=8/4	>=16	>=16	>=16	>=32	=<05)	<=8	>=16	<=025
Patient89	>=32	<= 8/4	>=16	>=16	>=16	>=32	<= 05	<= 8	>=16	2
Patient90	<=16	>=16/8	>=16	>=16	<= 18	32	4	>=8	>=16	>2
Patient91	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<= 8	>=16	>2
Patient92	<=16	>=16/8	>=16	>=16	>=16	>=32	<=025	<= 8	>=16	<=025
Patient93	<=16	16/8	>=16	>=16	>=16	>=32	4	<= 8	>=16	>2
Patient94	<=16	16/8	>=16	>=16	16	>=32	<=05	<=8	16	<=025
Patient95	<=16	>=16/8	>=16	>=16	>=16	>=32	<=05	<=8	16	<=025
Patient96	<=16	<=8/4	>=16	>=16	>=16	>=32	4	>=8	>=16	<=025
Patient97	>=32	>=16/8	>=16	>=16	>=16	>=32	<=05	<=8	4	<=025
Patient98	<=16	16/8	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient99	<=16	>=16/8	>=16	>=16	>=16	>=32	<=05	<=8	16	<=025
Patient100	<=16	>=16/8	>=16	>=16	>=16	>=32	4	>=8	16	>2
Patient101	>=32	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	<=1	<=025
Patient102	>=32	<=8/4	>=16	>=16	<= 18	>=32	<=05	<=8	8	>2
Patient103	<=16	>=16/8	>=16	>=16	>=16	>=32	<=05	<=8	16	<=025
Patient104	<=16	16/8	>=16	>=16	16	>=32	<=05	<=8	16	<=025
Patient105	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	16	<=025
Patient106	<=16	16/8	>=16	>=16	16	>=32	<=05	<=8	16	<=025
Patient107	>=32	<=8/4	>=16	>=16	>=16	>=32	4	<=8	>=16	>2
Patient108	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	8	<=025
Patient109	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	2
Patient110	<=16	<=16/8	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=0-25
Patient111	<=16	16/8	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025

Patient112	<=16	16/8	>=16	>=16	>=16	>=32	<=05	<=8	16	<=025
Patient113	<=16	<=8/4	<=8	<=8	<=8	>=32	<=05	<=8	4	>2
Patient114	<=16	16/8	>=16	>=16	16	>=32	<=05	<=8	4	<=025
Patient115	<=16	16/8	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient116	<=16	<=8/4	>=16	>=16	>=16	>=32	>=4	<=8	>=16	<=025
Patient117	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	16	<=025
Patient118	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	16	<=025
Patient119	<=16	<=8/8	>=16	>=16	<=8	8	<=05	<=8	<=1	<=025
Patient120	<=16	16/8	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient121	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	8	<=025
Patient122	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	16	<=025
Patient123	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	>=16	<=025
Patient124	<=16	16/8	>=16	>=16	>=16	>=32	<=05	>=8	8	<=025
Patient125	<=16	<=8/4	>=16	>=16	>=16	>=32	<=05	<=8	8	<=025
Patient126	<=16	16/8	>=16	>=16	>=16	>=32	<=05	<=8	16	<=025
Patient127	>=32	<=8/4	>=16	>=16	>=16	>=32	<=05	,=8	>=16	<=025
Patient128	<=16	16/8	<=16	<=16	<=16	>=32	<=05	<=8	>=16	<=025
Patient129	<=16	8/4	<=8	<=8	<=8	>=32	<=05	<=8	4	>2
Patient130	<=32	16/8	<=16	<=16	<=16	>=32	<=05	<=8	>=16	<=025
Patient131	<=16	16/8	<=16	<=16	<=16	>=32	4	>=8	>=16	2
Patient132	<=16	<=8/4	<=16	<=16	<=16	>=32	<=05	<=8	>=16	<=025
Patient133	<=16	16/8	<=16	<=16	<=16	>=32	4	>=8	>=16	>2
Patient134	<=16	16/8	<=16	<=16	<=16	>=32	<=05	<=8	>=16	<=025
Patient135	<=16	<=8/4	<=16	<=16	<=16	>=32	<=05	<=8	>=16	<=025
Patient136	<=16	<=8/4	<=16	<=16	<=16	>=32	<=05	<=8	>=16	<=025
Patient137	<=16	16/8	<=16	<=16	<=8	<=2	4	<=8	>=16	2
Patient138	<=16	16/8	<=16	<=16	<=16	>=32	<=05	<=8	>=16	<=025
Patient139	<=16	16/8	<=16	<=16	<=16	>=32	4	>=8	>=16	2
Patient140	<=16	<=8/4	<=16	<=16	<=16	>=32	<=05	>=8	>=16	<=025
Patient141	<=16	<=8/4	<=16	<=16	<=16	>=32	<=05	<=8	4	<=025
Patient142	<=16	<=8/4	<=16	<=8	<=8	8	<=05	<=8	<=1	<=025
Patient143	<=16	16/8	<=16	<=16	<=16	>=32	4	>=8	>=16	>2
Patient144	<=16	16/8	<=16	<=16	<=16	>=32	<=05	<=8	>=16	2
Patient145	<=16	16/8	<=16	<=16	<=16	>=32	<=05	>=8	>=16	2
Patient146	<=16	<=8/4	<=16	<=16	<=16	>=32	<=05	<=8	8	<=025

Table 1: *Multidrug resistance in bacteria and minimal inhibitory concentration (MIC).*

Treatment →	**Amikacin**	**Amox/K Calv**	**Ampicillin**	**Cefazolin**	**Cefepime**	**Cefotaxime**	**Cefotaxime/K Calvulanta**	**Cefoxitin**	**Ceftazidime**	**Ceftazidime/K Calvulantae**	**Pooled Total**
Observations No of Patients	146	146	146	146	146	146	146	146	146	146	1460
Sum $\sum xi$	2,594.0000	1,720.0000	2,304.0000	2,256.0000	2,142.0000	4,376.5000	158.7500	1,156.0000	1,936.0000	105.7500	18,749.0000
Mean $\underline{x}$	17.7671	11.7808	15.7808	15.4521	14.6712	29.9760	1.0873	7.9178	13.2603	0.7243	12.8418
Sum of squares $\sum x^2 i$	50,692.0000	22,720.0000	37,120.0000	35,712.0000	33,036.0000	138,896.2500	429.1875	9,232.0000	29,076.0000	386.9375	357,300.3750
Sample variance *s2*	31.7523	16.9447	5.2482	5.8770	11.1050	53.1460	1.7695	0.5449	23.4766	2.1403	79.8697
Sample std. dev. *s*	5.6349	4.1164	2.2909	2.4243	3.3324	7.2901	1.3302	0.7382	4.8453	1.4630	8.9370
Std. dev. of mean $SE\underline{x}$	0.4663	0.3407	0.1896	0.2006	0.2758	0.6033	0.1101	0.0611	0.4010	0.1211	0.2339

Table 2: *Descriptive statistics of k=10 independent treatments.*

Source	Sum of squares SS	Degrees of freedom ν	Mean square MS	F statistic	p-value
Treatment	94,489.1834	9	10,498.7982	690.6903	1.1102e-16
Error	22,040.6430	1450	15.2004		
Total	116,529.8264	1459			

Table 3: *One-way ANOVA of k = 10 independent treatments.*

Tukey HSD test

The p-value corresponding to F-statistic through one-way ANOVA was less than 0.01, which indicates that such or even more treatment pairs are substantially different. We have a set of pair treatments $k = 10$ in which Tukey's HSD test was added to every one of the ~45 pairs to determine which of them seem to have a statistically important difference. During the first time, the Tukey-Kramer HSD Q statistic's critical value was calculated based on treatments $k = 10$ and $n = 1450$ coefficients for the error phrase and significance level α = 0.01 and 0.05 (p-values) for the standardized residuals spectrum distribution. These critical values are obtained for Q, 0.01 and 0.05 as = 5.1682 and = 4.4812, respectively. These critical values could be checked in several published tables of the inverse predicted values range distribution, including this table at Duke University [12]. These critical values might be verified at several published tables of the inverse studentized range distribution, such as this table at Duke University [12]. Based on our microbial inhibition concentration (MIC) of each patient, samples were used in the Tukey test statistic to compare the appropriate critical value of the studentized range distribution. Tukey-Kramer 's confidence, which has been followed by the guidance of the NIST Engineering Statistics Handbook, has resulted in simplified algebraic transformations. We determined the factor for each pair of MIC columns as examined, which we roughly call the Tukey-Kramer HSD QQ-statistics here, and based on the Tukey HSD QQ-statistic equations, as follows

$$Q_{i,j} = \frac{|\bar{x}_i - \bar{x}_j|}{s_{i,j}}$$

In which the standard deviation in the expression as the follows

$$s_{i,j} = \frac{\hat{\sigma}_\epsilon}{\sqrt{H_{i,j}}} \quad i, j = 1,\ldots,k; i \neq j.$$

The proportion $H_{i,j}$ was the correlation coefficient of the number of data points in columns i and j where even the sampling frame sizes in the columns are the same. The harmonic standard error was essentially the expected probability sampling size. The appropriate harmonic mean has been necessary to apply the Tukey-Kramer method for columns with inequalities sample sizes. Quantity =3.8988 was also the square root of the Mean Square Error = 15.2004 calculated in the vital component one-way ANOVA method that had been the same across all pairs compared. The only other factor that varied across pairs throughout the data proc $s_{i,j} = \frac{\hat{\sigma}_\epsilon}{\sqrt{H_{i,j}}}$ was the square root, which was the harmonic mean of the contrasted sample sizes.

The assessment as to whether the NIST Tukey-Kramer correlation coefficient contains zero had also been compared to the assessment of whether $Q_{i,j} > Q_{critical}$ the was already calculated based on the optimum consistency of significance a (p-value), the number of treatments (k), and the degree of freedom for error (v) as indicated above.

Post-hoc Tukey HSD Test the results are calculated as follows

Range of $k = 10$ additional antibiotics treatments its degree of freedom for the term of inaccuracy $n = 1450$

Critical values for the residual range Q statistic

$Q_{critical}^{a=0.01,k=10,n=1450}$ = 5.1682 $Q_{critical}^{a=0.05,k=10,n=1450}$ = 4.4812

Significant results seemed to be color-coded (red to insignificant, unhighlighted to significant) in assessing whether $Q_{i,j} > Q_{critical}$ all treatment pairs had been appropriate (Table 4 A). Besides, we also analyzed the significance (p-value) of the Q-statistics $Q_{i,j}$ detected. The algorithm calculated the studentized range distribution's critical values and p-values corresponding to Gleason's data point $Q_{i,j}$ (1999). It was an enhancement and over Copenhaver-Holland (1988) algorithm used in the R statistical package.

Treatment's pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
Amikacin vs B Amox/K Calv	18.5527	0.0010053	** p < 0.01
Amikacin vs Ampicillin	6.1559	0.0010053	** p < 0.01
Amikacin vs Cefazolin	7.1748	0.0010053	** p < 0.01
Amikacin vs Cefepime	9.5948	0.0010053	** p < 0.01
Amikacin vs Cefotaxime	37.8377	0.0010053	** p < 0.01
Amikacin vs Cefotaxime/K Calvulanta	51.6939	0.0010053	** p < 0.01
Amikacin vs Cefoxitin	30.5249	0.0010053	** p < 0.01
Amikacin vs Ceftazidime	13.9676	0.0010053	** p < 0.01
Amikacin vs Ceftazidime/K Calvulantae	52.8189	0.0010053	** p < 0.01
Amox/K Calv vs Ampicillin	12.3968	0.0010053	** p < 0.01
Amox/K Calv vs Cefazolin	11.3779	0.0010053	** p < 0.01
Amox/K Calv vs Cefepime	8.9579	0.0010053	** p < 0.01
Amox/K Calv vs Cefotaxime	56.3904	0.0010053	** p < 0.01
Amox/K Calv vs Cefotaxime/K Calvulanta	33.1412	0.0010053	** p < 0.01
Amox/K Calv vs Cefoxitin	11.9722	0.0010053	** p < 0.01
Amox/K Calv vs Ceftazidime	4.5851	0.0400370	* p < 0.05
Amox/K Calv vs Ceftazidime/K Calvulantae	34.2662	0.0010053	** p < 0.01
Ampicillin vs Cefazolin	1.0189	0.8999947	Insignificant
Ampicillin vs Cefepime	3.4388	0.3078406	Insignificant
Ampicillin vs Cefotaxime	43.9936	0.0010053	** p < 0.01
Ampicillin vs Cefotaxime/K Calvulanta	45.5379	0.0010053	** p < 0.01
Ampicillin vs Cefoxitin	24.3690	0.0010053	** p < 0.01
Ampicillin vs Ceftazidime	7.8117	0.0010053	** p < 0.01
Ampicillin vs Ceftazidime/K Calvulantae	46.6630	0.0010053	** p < 0.01
Cefazolin vs Cefepime	2.4199	0.7623243	Insignificant
Cefazolin vs Cefotaxime	45.0126	0.0010053	** p < 0.01
Cefazolin vs Cefotaxime/K Calvulanta	44.5190	0.0010053	** p < 0.01

Cefazolin vs Cefoxitin	23.3501	0.0010053	** p < 0.01
Cefazolin vs Ceftazidime	6.7927	0.0010053	** p < 0.01
Cefazolin vs Ceftazidime/K Calvulantae	45.6441	0.0010053	** p < 0.01
Cefepime vs Cefotaxime	47.4325	0.0010053	** p < 0.01
Cefepime vs Cefotaxime/K Calvulanta	42.0991	0.0010053	** p < 0.01
Cefepime vs Cefoxitin	20.9302	0.0010053	** p < 0.01
Cefepime vs Ceftazidime	4.3728	0.0628677	Insignificant
Cefepime vs Ceftazidime/K Calvulantae	43.2242	0.0010053	** p < 0.01
Cefotaxime vs Cefotaxime/K Calvulanta	89.5316	0.0010053	** p < 0.01
Cefotaxime vs Cefoxitin	68.3626	0.0010053	** p < 0.01
Cefotaxime vs Ceftazidime	51.8053	0.0010053	** p < 0.01
Cefotaxime vs Ceftazidime/K Calvulantae	90.6566	0.0010053	** p < 0.01
Cefotaxime/K Calvulanta vs Cefoxitin	21.1690	0.0010053	** p < 0.01
Cefotaxime/K Calvulanta vs Ceftazidime	37.7263	0.0010053	** p < 0.01
Cefotaxime/K Calvulanta vs Ceftazidime/K Calvulantae	1.1250	0.8999947	Insignificant
Cefoxitin vs Ceftazidime	16.5573	0.0010053	** p < 0.01
Cefoxitin vs Ceftazidime/K Calvulantae	22.2940	0.0010053	** p < 0.01
Ceftazidime vs Ceftazidime/K Calvulantae	38.8513	0.0010053	** p < 0.01

Table 4A: *Tukey HSD test relationship between statistically significant comparisons for two sets of antibiotics.*

Scheffé multi comparative analysis

As stated in the same National Institute of Standards and Technology (NIST) Engineering Statistics Handbook page for Scheffe's Method [13], we characterize a statistic labeled *T* as the ratio of unsigned comparison to standard error. It has been shown that for comparisons that are treatment pair (i, j) of unit coefficients,

$$T_{i,j} = \frac{Q_{i,j}}{\sqrt{2}}$$

Where $Q_{i,j}$ was indeed the Q-statistic generated and for the Tukey HSD test. This T-statistic has exciting characteristics.

The same valid of the NIST Engineering Statistics Textbook page for Scheffé hand calculations as for the provision of a formula that leads directly to the Scheffe p-value related to the reported T-value as:

$$1 - F\left(\frac{T^2}{k-1}, k-1, \nu\right)$$

Where F was the cumulative distribution of F including its two degrees of freedom parameters $k \square 1$ and n. Consider that k was the range of treatments and n was the degree of freedom of error mentioned in the previous section.

The Scheffé p-value of the examined T-statistic $T_{i,j}$ was shown in below all appropriate pairs of treatments along with all the Scheffé color-coded interpretation (red for negligible, not highlighted for significant) significant with a p-value (Table 4 B).

Treatment's pair	Scheffé TT-statistic	Scheffé p-value	Scheffé inference
Amikacin vs Amox/K Calv	13.1187	1.1102e-16	** p < 0.01
Amikacin vs Ampicillin	4.3529	0.0263181	* p < 0.05
Amikacin vs Cefazolin	5.0734	0.0023970	** p < 0.01
Amikacin vs Cefepime	6.7845	7.7039e-07	** p < 0.01
Amikacin vs Cefotaxime	26.7553	1.1102e-16	** p < 0.01
Amikacin vs Cefotaxime/K Calvulanta	36.5531	1.1102e-16	** p < 0.01
Amikacin vs Cefoxitin	21.5844	1.1102e-16	** p < 0.01
Amikacin vs Ceftazidime	9.8766	2.2204e-16	** p < 0.01
Amikacin vs Ceftazidime/K Calvulantae	37.3486	1.1102e-16	** p < 0.01
Amox/K Calv vs Ampicillin	8.7658	1.5428e-12	** p < 0.01
Amox/K Calv vs Cefazolin	8.0454	2.8514e-10	** p < 0.01
Amox/K Calv vs E	6.3342	8.7101e-06	** p < 0.01
Amox/K Calv vs Cefotaxime	39.8740	1.1102e-16	** p < 0.01
Amox/K Calv vs Cefotaxime/K Calvulanta	23.4343	1.1102e-16	** p < 0.01
Amox/K Calv vs Cefoxitin	8.4656	1.4422e-11	** p < 0.01
Amox/K Calv vs Ceftazidime	3.2422	0.3116230	Insignificant
Amox/K Calv vs Ceftazidime/K Calvulantae	24.2299	1.1102e-16	** p < 0.01
Ampicillin vs Cefazolin	0.7205	0.9999639	Insignificant
Ampicillin vs Cefepime	2.4316	0.7483968	Insignificant
Ampicillin vs Cefotaxime	31.1082	1.1102e-16	** p < 0.01
Ampicillin vs Cefotaxime/K Calvulanta	32.2002	1.1102e-16	** p < 0.01
Ampicillin vs Cefoxitin	17.2315	1.1102e-16	** p < 0.01
Ampicillin vs Ceftazidime	5.5237	0.0003951	** p < 0.01
Ampicillin vs Ceftazidime/K Calvulantae	32.9957	1.1102e-16	** p < 0.01
Cefazolin vs Cefepime	1.7111	0.9669262	Insignificant
Cefazolin vs Cefotaxime	31.8287	1.1102e-16	** p < 0.01
Cefazolin vs Cefotaxime/K Calvulanta	31.4797	1.1102e-16	** p < 0.01
Cefazolin vs Cefoxitin	16.5110	1.1102e-16	** p < 0.01
Cefazolin vs Ceftazidime	4.8032	0.0063228	** p < 0.01
Cefazolin vs Ceftazidime/K Calvulantae	32.2752	1.1102e-16	** p < 0.01
Cefepime vs Cefotaxime	33.5398	1.1102e-16	** p < 0.01
Cefepime vs Cefotaxime/K Calvulanta	29.7686	1.1102e-16	** p < 0.01
Cefepime vs Cefoxitin	14.7999	1.1102e-16	** p < 0.01
Cefepime vs Ceftazidime	3.0921	0.3879182	Insignificant
Cefepime vs Ceftazidime/K Calvulantae	30.5641	1.1102e-16	** p < 0.01
Cefotaxime vs Cefotaxime/K Calvulanta	63.3084	1.1102e-16	** p < 0.01
Cefotaxime vs Cefoxitin	48.3397	1.1102e-16	** p < 0.01
Cefotaxime vs Ceftazidime	36.6319	1.1102e-16	** p < 0.01
Cefotaxime vs Ceftazidime/K Calvulantae	64.1039	1.1102e-16	** p < 0.01
Cefotaxime/K Calvulanta vs Cefoxitin	14.9687	1.1102e-16	** p < 0.01
Cefotaxime/K Calvulanta vs Ceftazidime	26.6765	1.1102e-16	** p < 0.01
Cefotaxime/K Calvulanta vs Ceftazidime/K Calvulantae	0.7955	0.9999160	Insignificant
Cefoxitin vs Ceftazidime	11.7078	1.1102e-16	** p < 0.01
Cefoxitin vs Ceftazidime/K Calvulantae	15.7642	1.1102e-16	** p < 0.01
Ceftazidime/K Calvulantae vs Ceftazidime	27.4720	1.1102e-16	** p < 0.01

***Table 4b**: Scheffe test statistic, multiple comparisons and critical value.*

Multiple similarities between Bonferroni and Holm

Having similar statistic T for its Scheffé system, along with the total of contrasts (pairs) q being contrasted simultaneously, contributes to the Bonferroni equation. Further the Bonferroni method contains a formula that leads directly to the Bonferroni p-value relating to the reported T-value in the range of the parallel comparison of q contrasts as follows

Bonferroni p-value: $P_{i,j}^{Bonferroni} = P_{i,j}^{unadjusted} q$

where

$$P_{i,j}^{unadjusted} = \left[1 - t\left(\frac{T^2}{k-1}, v\right)\right] 2$$

And were t with its degree of freedom parameter v was the cumulative t distribution. Noted that v was the degrees of freedom of error that had already been defined. Confirm also that Bonferroni's p-value simultaneous relation was strictly proportional to q, compared to the number of contrasts (pairs) continuously. The Holm method listed in Aickin and Gensler's (1996) [11] review paper involves sorting the $P_{i,j}^{unadjusted}$ in ascending order above and calculating $R_{i,j}$ each unique pair (i, j). This form of rank varies from 1 to q. In the sense of several comparisons of q such pairs simultaneously, the Holm p-value for comparing a given pair (i, j) was:

P-value of Holm: $P_{i,j}^{Holm} = P_{i,j}^{unadjusted}\left(q - R_{i,j} + 1\right)$

We consider all possible contrasts of unique antibiotics for combined comparison in this first combined Bonferroni and Holm table below, so $q = 45$. For all appropriate $q = 45$ pairs of treatments, the Bonferroni and Holm p-values of even the reported T-statistic were shown below and the color-coded Bonferroni and Holm inferences (red for insignificant, un-highlighted for significant) known as the p-value (Table 4 C).

Treatment's pair	Bonferroni and Holm TT-statistic	Bonferroni p-value	Bonferroni inference	Holm p-value	Holm Inference
Amikacin vs Amox/K Calv	13.1187	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Amikacin vs Ampicillin	4.3529	0.0006469	** p < 0.01	0.0001006	** p < 0.01
Amikacin vs Cefazolin	5.0734	1.9860e-05	** p < 0.01	3.9720e-06	** p < 0.01
Amikacin vs Cefepime	6.7845	7.6127e-10	** p < 0.01	2.0301e-10	** p < 0.01
Amikacin vs Cefotaxime	26.7553	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Amikacin vs Cefotaxime/K Calvulanta	36.5531	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Amikacin vs Cefoxitin	21.5844	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Amikacin vs Ceftazidime	9.8766	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Amikacin vs Ceftazidime/K Calvulantae	37.3486	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Amox/K Calv vs Ampicillin	8.7658	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Amox/K Calv vs Cefazolin	8.0454	7.9936e-14	** p<0.01	2.3093e-14	** p < 0.01
Amox/K Calv vs Cefepime	6.3342	1.4290e-08	** p < 0.01	3.4930e-09	** p < 0.01
Amox/K Calv vs Cefotaxime	39.8740	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Amox/K Calv vs Cefotaxime/K Calvulanta	23.4343	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01

Amox/K Calv vs Cefoxitin	8.4656	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Amox/K Calv vs Ceftazidime	3.2422	0.0545961	Insignificant	0.0072795	** p < 0.01
Amox/K Calv vs Ceftazidime/K Calvulantae	24.2299	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Ampicillin vs Cefazolin	0.7205	21.2105651	Insignificant	0.4713459	Insignificant
Ampicillin vs Cefepime	2.4316	0.6818380	Insignificant	0.0606078	Insignificant
Ampicillin vs Cefotaxime	31.1082	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Ampicillin vs Cefotaxime/K Calvulanta	32.2002	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Ampicillin vs Cefoxitin	17.2315	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Ampicillin vs Ceftazidime	5.5237	1.7682e-06	** p < 0.01	3.9293e-07	** p < 0.01
Ampicillin vs Ceftazidime/K Calvulantae	32.9957	0.0000e+00	** p<0.01	0.0000e+00	** p < 0.01
Cefazolin vs Cefepime	1.7111	3.9271195	Insignificant	0.2618080	Insignificant
Cefazolin vs Cefotaxime	31.8287	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Cefazolin vs Cefotaxime/K Calvulanta	31.4797	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Cefazolin vs Cefoxitin	16.5110	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Cefazolin vs Ceftazidime	4.8032	7.7548e-05	** p < 0.01	1.3786e-05	** p < 0.01
Cefazolin vs Ceftazidime/K Calvulantae	32.2752	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Cefepime vs Cefotaxime	33.5398	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Cefepime vs Cefotaxime/K Calvulanta	29.7686	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Cefepime vs Cefoxitin	14.7999	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Cefepime vs Ceftazidime	3.0921	0.0911537	Insignificant	0.0101282	* p < 0.05
Cefepime vs Ceftazidime/K Calvulantae	30.5641	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Cefotaxime vs Cefotaxime/K Calvulanta	63.3084	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Cefotaxime vs Cefoxitin	48.3397	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Cefotaxime vs Ceftazidime	36.6319	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Cefotaxime vs Ceftazidime/K Calvulantae	64.1039	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Cefotaxime/K Calvulanta vs Cefoxitin	14.9687	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Cefotaxime/K Calvulanta vs Ceftazidime	26.6765	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Cefotaxime/K Calvulanta vs Calvulantae	0.7955	19.1896081	Insignificant	0.8528715	Insignificant
Cefoxitin vs Ceftazidime	11.7078	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Cefoxitin vs Ceftazidime/K Calvulantae	15.7642	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Ceftazidime vs /K Calvulantae	27.4720	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01

Table 4C: *Bonferroni and Holm results: all pairs simultaneously compared.*

We suggest a subset of contrasts (sets of various antibiotics) in this second Bonferroni and Holm table below for a simultaneous comparison of only pairs compared to treatment with Amikacin antibiotics. Where the care of the first column of antibiotics is regulated, such a condition could be significant. The experimenter was only interested in disparities in treatment as compared to control, thus $q = 9$. Bonferroni and Holm p-values of the T-statistics $T_{i,j}$ obtained for $q = 9$ relevant treatment pairs, along with color-coded Bonferroni inference dependent on p-value (red for insignificant, un-highlighted for significant) (Table 5).

Treatment's pair	Bonferroni and Holm TT-statistic	Bonferroni p-value	Bonferroni inference	Holm p-value	Holm inference
Amikacin vs Amox/K Calv	13.1187	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Amikacin vs Ampicillin	4.3529	0.0001294	** p < 0.01	1.4377e-05	** p < 0.01
Amikacin vs Cefazolin	5.0734	3.9720e-06	** p < 0.01	8.8266e-07	** p < 0.01
Amikacin vs Cefepime	6.7845	1.5225e-10	** p < 0.01	5.0751e-11	** p < 0.01
Amikacin vs Cefotaxime	26.7553	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Amikacin vs Cefotaxime/K Calvulanta	36.5531	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Amikacin vs Cefoxitin	21.5844	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Amikacin vs Ceftazidime	9.8766	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01
Amikacin vs Ceftazidime/K Calvulantae	37.3486	0.0000e+00	** p < 0.01	0.0000e+00	** p < 0.01

Table 5: *Bonferroni and Holm results: only pairs relative to a simultaneously compared.*

Tested Tukey HSD post-hoc calculation

Microsoft Excel retains built-in functions related to the distribution of the Studentized spectrum, so even though the Mean Square Error measured it in one-way ANOVA, whose square root was $\hat{\sigma}_{\epsilon}$, and was conscious of all the sample sizes and degrees of freedom, one more step in the comparison of treatments was unable to execute the post-hoc Tukey HSD. To directly conduct the post-hoc Tukey HSD test calculation to take the mean squared error from the one-way ANOVA output of Excel and then to calculate its square root $\hat{\sigma}_{\epsilon}$. Finally, break $\hat{\sigma}_{\epsilon}$ this by the square root $H_{i,j}$ of the harmonic mean of the respective columns of the sample being compared, resulting $S_{i,j}$ in columns (i, j) for each pair. Excel has a built-in HARMEAN (*n1, n2*) function that measures the harmonic mean value. These are based on determining $Q_{i,j} = \frac{|\bar{x}_i - \bar{x}_j|}{S_{i,j}}$ outcomes. For the estimation of the numerator, Microsoft Excel supplies the corresponding sample column averages (means). Finally, compare whether $Q_{i,j} > Q_{critical}$ other critical values for the required number of degrees of freedom of error and the number of treatments based almost entirely on the studentized spectrum are obtained for this comparison.

Verify Scheffé, Bonferroni, and Holm post-hoc calculations

For the steps of Scheffé, Bonferroni, and Holm, calculate and separate the T-statistic $T_{i,j}$ for all pairs $Q_{i,j}$ from in the intermediate phase $\sqrt{2}$ of Tukey HSD. Measure the Scheffé p-value of the T-statistic obtained by the built-in function for the F distribution used by Excel, which also has the F.DIST form (x, deg freedom1, deg freedom2, cumulative). Set x as $\frac{T^2}{k-1}$, to the first statement, $k = 1$ and v was the second and third statements. The fourth point was set at 1. In Excel, the Scheffé p-value of the formula was determined as 1-F. ($x, k = 1, v, 1$) DIST. For the comparison of Bonferroni and Holm, consider taking the same T-statistic $T_{i,j}$, which was calculated above for the Scheffé step. Ascertain q, the number of pairs, which are compared separately. Using the Excel built-in function for the t-distribution T. DIST (x, deg freedom, cumulative) to measure the Bonferroni p-value of its reported T-statistic. In Excel, the formula measures the seasonally adjusted p-value $P^{unadjusted}$ (1-T. DIST (T, v, TRUE)) *2. For each pair, the Bonferroni p-value was estimated (i, j) as $P^{Bonferroni} = P^{unadjusted} q$ to evaluate each given pair's (i, j) sort rank $R_{i,j}$, and the q-element array $P^{unadjusted}_{i,j}$

was sorted in ascending order. This form of rank varies from 1 to q. For each pair, the Holm p-value was determined for (i, j) us $P_{i,j}^{Holm} = P_{i,j}^{unadjusted}\left(q \square R_{i,j} + 1\right)$.

Discussions

Antibiotic-resistant bacteria investigation forms an integral part of identifying and improving initiatives to regulate the spread of resistance between bacterial isolates. In that same study, *Escherichia coli*, Proteus mirabilis, *Klebsiella pneumoniae*, *Klebsiella aerogenes*, and *Enterobacter cloacae* are significant bacteria isolated outpatients in the Saudi Arabia hospitals in the Makkah zone. The results indicate that antibacterial resistance among health center isolates is extensive. However, the amount of resistance was smaller than in the tertiary hospitals of the downtown areas [14]. This research aimed to test the extent of the significance of AMR among study participants in three separate Makkah hospitals. The issue of antimicrobial resistance applies to the impact of incidents that would not have existed if resistance were not present. Such result indicators can include increased risk of death, hospital stay sickness, accidents, and potential due mainly costs. The risk of such damaging impacts is higher in patients with antibiotic-resistant infections in different microorganisms compared to infections caused by pathogenic strains of the same pathogen compared to infections caused by susceptible strains of the same pathogen [15,16], even after regulation for multiple underlying medical conditions at the same time, primarily when they associate with each other in some way [17]. As recorded, only 26.85% of the respondents were aware of antibiotic resistance, which is very limited relative to the findings of a related study conducted in other parts of the kingdom [18].

A higher percentage of AMR information was recorded from South Korea (Kim et al., 2011), more than one-third (36%) of Kuwait (Award et al., 2015), Saudi Arabia, and Jordan (60.7%) concerning this study (Shehadeh et al., 2012). Moreover, as opposed to Hong Kong (91%) and Indonesia (85%), respondents had more excellent knowledge of the term antibiotic resistance (Widayati et al., 2012). The analyses discussed above-revealed differences in the awareness of communities in various regions about the AMR. Overall, 70% of respondents in all countries surveyed said they had used the word before, the highest degree of understanding of antibiotic resistance, and closely followed by antibiotic resistance (68%) and antibiotic-resistant (66%) of bacteria. The least familiar (21%) is AMR. Almost fourteen (World Health Organization 2015) is the percentage of all people surveyed who have never heard any words.

No major significant differences in the antimicrobials used in three separate hospitals have been found. We are also based on this important health care treatment center, where samples are often routinely sent for pathogen detection from other hospitals. In each patient screened with various antibiotics studied in microorganisms, the average antibiotic resistance profiling determines minimum inhibitory concentration tests. Still, the important test by statistical analysis was non-hypothesis provided a significant value of p=1.1102e-16 out of ~46 patients tested with ten different antibiotics. Besides, pair treatments were matched between the two different antibiotics determined by ANOVA Tukey HSD calculations. Table 4 shows each pair set of treatments with antibiotics given most of the significant (p<0.05). For Escherichia coli was high levels of resistance to Ampicillin (146/146) 100% (p<1.0), Cefepime (146/146) 100% (p<1.0), and Cefazolin (145/146) 99.31% (p<0.97) (Figures 3 and 4) with susceptible to be indicated a typical pattern of initial treatment (Table 1). The major ESBL-producing species worldwide are Klebsiella pneumoniae and Escherichia coli (Bush et al., 2010; Shakya et al., 2017). Both these organisms will also lead to the propagation of ESBL in healthcare settings. ESBL often are resistant also to Cefotaxime and Ceftazidime. Therefore, ESBL also shows a phenotype of multi-drug resistance and is an actual cause of lack of treatment (Bush et al., 2011). Non-beta-lactam resistances have been reported in various years' in-group variants of MRSA, especially USA300 (Chua et al., 2011; Chadwick et al., 2013). We investigated the prevalence rate of ESBL-producing strains from Saudi Arabia and observed that the occurrence of ESBLs in Klebsiella pneumoniae was 48.4%, followed by 15.8% in E. coli (El-Khizzi et al., 2006). E. coli, 24.8%, and 30.5% of K. Pneumoniae were positive for Saudi Arabia's recorded output of ESBLs (Shibl et al., 2012; Aldrazi et al., 2020). Bacterial resistance induced by ESBL has been recognized as a real health problem. Even limited data on ESBL prevalence and molecular characterization in hospitals in the Makkah region of Saudi Arabia is currently available.

Conclusions

Thus, during 2016, we examined antimicrobial resistance to four distinct microbial pathogens in three leading hospitals in this research. While the *E. coli* resistant profiles of Ampicillin, Cefepime, and Cefazolin were dominant, Amoxicillin and potassium clavulanate and Cefoxitin were also adequate. The elevated multi-drug resistance concentrations of Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Enterobacter cloacae, and Klebsiella aerogenes have been of significant concern. As there was an increased risk of possible multi-drug resistance to ESBL, the therapeutic potential proved safe.

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Protection of Human and Animal Subjects

The authors declare that the procedures followed the relevant clinical research ethics committee's regulations and those of the world medical association code of ethics (Declaration of Saied Al-Dehlawi).

Confidentiality of Data

The authors confirm that they have pursued the guidelines of their work center for the publishing of patient results.

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All authors: No reported conflicts of interest. All authors have submitted form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Author Contributions

S.D., Z.A., and S.M designed research contributed new reagents. Analytic tools data analysis; K.A. Analyzed data; S.D., Z.A., and K.A. wrote the paper and A.K. reviewed the manuscript. Principle Investigator; S.D.

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