Original article

Study of antimicrobial susceptibility of clinically significant microorganisms isolated from selected areas of Dhaka, Bangladesh.

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Abstract

Objective: Pathogenic resistance against antibiotics is substantially mounting in the developing countries including Bangladesh. Present study thus attempted to obtain the baseline information on such resistance among the community people coming to the local dispensaries around the city of Dhaka for treatment. ***Materials and Methods***: A total of 2,700 clinical specimens were examined for the presence of Gram positive and Gram negative pathogens. Antibiotic susceptibility tests of the isolates were carried out. Extended spectrum b- lactamase (ESBL) activity, and the presence of methicillin resistant *Staphylococcus aureus* (MRSA) and *S. epidermidis* (MRSE) were also detected. ***Results****: Escherichia coli* were most prevalent (45.5%) among 1044 pathogenic bacteria isolated from 2,700 samples. *E. coli* predominated urine, pus, wound swab, blood, high vaginal swab (HVS) and sputum specimens, and exhibited the highest frequency of ESBL activity (35%). Prevalence of *Klebsiella* spp. and *S. aureus* among the clinical specimens were 11.5% and 9.86%, respectively. Most of the Gram negative bacilli were found resistant against ciprofloxacin (5 mg), tetracycline (30 mg) and cotrimoxazole (25 mg). Majority of *Pseudomonas* spp. were found resistant against most of the commonly used antibiotics. Interestingly, around half of the *S. aureus* isolates were observed to be methicillin resistant, but not vancomycin resistant. ***Conclusion***: Overall, such a revelation of increased antibiotic resistance demands for restrictive and appropriate antibiotic usage in accordance with the updated antibiotic prescribing policy in Bangladesh.

Key words: Pathogens; drug resistance; Extended spectrum b- lactamase (ESBL); Methicillin resistant *Staphylococcus aureus* (MRSA); Methicillin resistant *S. epidermidis* (MRSE).

Introduction

Antibiotic resistance is a major clinical hindrance in treating infections caused by pathogenic microorganisms[1]. The bacterial resistance to antimicrobial agents is known to be driven by the interplay of several mechanistic and epidemiologic factors, including the chromosomal defects, random mutation, plasmid exchange, and by the transfer of drug resistance genes by intigron or transposon[2-5]. Besides, the widespread and indiscriminate use of antibiotics including the addition of antibiotics to livestock feed has led to the development of serious problems of resistance and hence limits the usefulness of antibiotics to eliminate bacterial infections[6-9].

In conjunction with such problems, in recent years, methicillin-resistant *Staphylococcus aureus* (MRSA) and the extended-spectrum ?-lactamase (ESBL) producing bacteria have been reported to be responsible for several difficulties to treat infections in humans [10, 11]. MRSA is considered to be any strain

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of *S. aureus* developing the resistance against the b-lactam antibiotics including penicillins and cephalosporins [10]. ESBLs are plasmid mediated enzymes capable of hydrolyzing and inactivating a wide variety of ?-lactam bearing antibiotics and have been widely reported to be found in both enteric and non-enteric bacteria [11, 12]. A study conducted by National Healthcare Network (NHN), Dhaka, showed that 43% of the isolated *S. aureus* were MRSA and 10-20% *Enterobacteriaceae* was ESBL producers [13-14].

The most important aspect of antibiotic resistance underlies on their irrational use, inappropriate self medication, and increased consumption of non-prescribed antibiotics led to the antibiotic ineffectiveness commonly, leading to an increase of morbidity and early mortality [15]. In Bangladesh, a recent study has shown that more than 70% of infecting bacteria were resistant against at least one of the antibiotics commonly used to treat [14]. It is noteworthy that the antibiotic resistance is not a static phenomenon and hence a regular updating of antibiogram is very essential for the judicious use of antibiotics. Moreover, the antibiotic susceptibility test contributes directly to patient care, and also may pose a significant impact on the bona fide usage of antibiotics. Thus, the acquaintance on the current susceptibility pattern is imperative for the physicians in order to select the appropriate antimicrobials and for developing the appropriate prescribing policies as well [16].

Along these lines, we primarily isolated the common and clinically significant pathogens around the city of Dhaka, with the subsequent detection of their antimicrobial susceptibility patterns, and recorded the data of drug resistance including the common ones. Such an attempt of data assembly, in turn, is expected to assist our physicians to select the effective antibiotic for appropriate medication as well as to introduce the change for the better management of the overall public health in Bangladesh.

Materials and Methods

Sample and sampling areas

The study was carried out in the National Healthcare Network (NHN) Microbiology Laboratory, Dhaka, during the time period of April 01, 2011 to March 30, 2012. A total of 2,700 clinical specimens including blood, sputum, stool, urine, pus, wound and throat swab samples were analyzed for the presence of pathogenic microorganisms. Specimens were collected from different sub-centers of NHN located at the North (Mirpur and Shyamoli) and South (Wari, Dhanmondi, Nawab Garden and Farashganj) zones of Dhaka city.

Isolation & identification of pathogenic bacteria

To isolate particularly concerned pathogenic microorganisms, all the specimens were spread on the MacConkey agar, blood agar, chocolate agar, and manitol salt agar media plates, and were incubated at 37 ^{o}C for 18 to 24 hours. Chocolate agar plates were incubated in CO_2 enriched jar at 37 ^{o}C, followed by subsequent observation for the appearance of characteristic colonies. Colony color, shape, elevation, surface texture, opacity, etc. on different media were recorded. For initial identification, the size, shape and arrangements of the suspected isolated colonies were observed by Gram staining under the bright field microscope at 1000× magnification. Finally, a series of biochemical tests were performed to identify the bacteria of interest following standard methods[17] including the triple sugar iron agar (TSI) test, motility indole urea (MIU) test, citrate utilization test, catalase test, oxidase test, coagulase test, bile esculin agar test and camp test.

Antibiotic susceptibility assay of bacterial isolates

Kirby-Bauer disc diffusion technique[18] recommended by the National Committee for Clinical Laboratory Standards (NCCLS) was applied to determine the bacterial susceptibility against different antibiotics used. The quality of the antibiotic discs were tested by using ATCC strains of *E. Coli* 25922 and *S. aureus* 25923, collected from the Laboratory of Bangladesh Institute of Research and Rehabilitation for Diabetes, Endocrine and Metabolic Disorders (BIRDEM) hospital. Amoxyclav (30 µg), ceftazidime (30 µg), cefexime (5 µg), cefuroxime (30 µg), ciprofloxacin (5 µg), cephalexin (30 µg), cotrimoxazole (25 µg), tetracycline (30 µg), amikacin (30 µg), gentamicin (10 µg), imipenem (10 µg), nitrofurantoin (300 µg), ampicillin (10 µg), oxacillin (1 µg), penicillin (10 µg), vancomycin (30 µg) and carbenecillin (100 µg) were used for the assay. Suspensions of the test organisms were prepared using Muller-Hinton broth by adjusting the turbidity of the broth with normal saline to match the equivalent turbidity standard of

McFarland (0.5 standards) and was incubated for 2 hours. Sterile cotton swabs were dipped into the suspensions and the swabs were then evenly spread over the entire surface of a Muller-Hinton agar plate to obtain uniform inoculums. Antibiotic discs of appropriate concentrations were applied aseptically over the surface of the inoculated plates at appropriate spatial arrangement by means of sterile needle within a distance of 5 mm. Plates were then inverted and incubated at 37 ^{o}C. After 24 hours, plates were examined and the diameters of the zones of complete inhibition were measured and interpreted as susceptible, intermediate and resistant [19].

Detection of extended spectrum b- lactamase (ESBL) by the double disc diffusion method

E. coli, *Enterobacter, Citrobacter* and *Klebsiella* were inoculated onto Mueller-Hinton agar media. Amoxyclav impregnated disc was then placed at the center of the Mueller-Hinton plate while ceftazidime, ceftriaxone, cefixime, cefuroxime discs were placed peripherally away from the amoxyclave disc. Band formation between amoxyclav disc and any other disc were considered as ESBL positive [14, 20].

Detection of methicillin resistant S. aureus (MRSA) and S. epidermidis (MRSE)

MRSA and MRSE were detected by testing susceptibility to oxacillin [14, 19]. Suspensions of *S. aureus* and *S. epidermidis* were inoculated onto Mueller-Hinton agar media. A disc containing 1 mg of oxacillin was placed over the inoculated media and incubated at 37 ^{o}C for 24 hours. Zone diameter of <10 mm referred to the oxacillin resistance and was regarded as MRSA and MRSE.

Results

Distribution of pathogens

Among the specimens where the growth of pathogenic bacteria were detected in our study, urine ranked first (71.93%) followed by pus (11.30%), wound swab (5.2%), sputum (4.78%) and blood (3.35%) samples. The frequency of isolated pathogenic bacteria were very low in case of conjunctival swab, throat swab, high vaginal swab (HVS) and stool samples (1.05%, 1.05%, 1.08% and 0.29%, consecutively). The most frequently isolated organisms from all samples were *E. coli* (45.50%), *Klebsiella* spp. (11.50%), *S. aureus* (9.86%) and *Pseudomonas* spp. (4.31%) (table I). *E. coli* was found to be the most common pathogen (56.46%) from urine samples followed by *Klebsiella* spp. (13.04%), *Enterococcus* spp. (4.93%) *and Streptococcus agalactiae* (4.93%). Among the pus and wound swab samples, *S. aureus* and *Pseudomonas* spp. were the most commonly isolated pathogens.

Antibiotic resistance patterns exhibited by Gram negative bacteria

In accordance to our stated objective of determining the antibiotic resistance pattern, a series of data was accumulated both for Gram negative and Gram positive bacteria (tables II, III and figure I). In our study, 89% isolates were found to be resistant against ciprofloxacin while 62% were resistant against amoxyclav (table II). Almost all Gram negative bacilli were found sensitive against imipenem. Among the ESBL producers, the prevalence of *E. coli*, *Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp. were measured to be 35%, 22%, 21% and 19%, consecutively.

Antibiotic resistance patterns exhibited by Gram positive bacteria

Among the *S. aureus* and *S. epidermidis* isolates, nearly half (46%) of the strains were MRSA while 30% were found to be MRSE. Two thirds of them were also resistant against ampicillin and mostly against the penicillins (table III). All of the isolated *Streptococcus pyogens* were found to be sensitive against penicillin, amoxyclave, ampicillin and oxacillin. On the other hand, in case of *Streptococcus agalactiae* isolates, the proportion of resistance ranged between 94 - 97% against ampicillin, amoxyclav and penicillin. The resistance patterns of *Enterococcus* spp. were observed to be satisfactory in penicillin and cephalosporin group. Interestingly, in our study, all of the isolated *S. aureus* showed sensitivity against vancomycin., i. e., vancomycin resistant *S. aureus* (VRSA) were completely absent.

Model of antibiotic resistance index

The resistance patterns exhibited both by Gram positive and Gram negative bacteria against the similar antibiotics were assembled as shown in figure I. From this model, the prevalence of commonly resistant pathogens is clearly evident. For example, both Gram positive (*S. aureus*, *S. epidermidis*, *Streptoccous agalactiae*, b-haemolytic streptococci) and Gram negative bacteria (*Citrobacter* spp.) could be seen to be resistant against cotrimoxazole. Other common resistance was visualized for ciprofloxacin

and tetracycline. Cephalexin and amoxyclav, on the other hand, were found effective against certain Gram positive pathogenic bacteria.

Discussion

Appropriate information on drug resistance is an essential concern for the formulation of antibiotic prescribing policy. In the present study, we portrayed the drug resistance patterns of the pathogens which further aided to construct a model of general antibiogram index. The isolates were found resistant against most of the commonly used antibiotics, thus steering the treatment strategy to failure. A significant point is to ponder across the study that 89% of the *Pseudomonas species* were found to be resistant against ciprofloxacin, a commonly used inexpensive oral antibiotic. Even a few years earlier, the proportion of the ciprofloxacin resistant *Pseudomonas* in Dhaka city has been reported to be much lesser than that of present time [14, 21-22]. Thus, the trend of antibiotic resistance has been found to be escalating through the updated approach of our study.

Multi-drug resistant Gram-positive bacteria including MRSA, MRSE, VRSA, methicillin-resistant coagulase-negative Staphycolocci (MRCNS), and penicillin-resistant *Streptococcus pneumoniae* (PRSP) are known to be a serious problem in the medical community [23-24]. A study carried out by Pakistan Armed Forces Institute of Pathology revealed that frequency of MRSA among all nosocomial isolates of *Staphylococcus aureus* increased from 39% in 1996 to 51% in 2003 [25]. The data relating MRSA and MRSE through our study also reveals the overall vulnerability against infections.

One important facet has been revealed through our study that we did not obtain any VRSA. Several reports have shown the prevalence of VRSA and Vancomycin Intermediate *S. aureus* (VISA) in India, Japan, United States, France, United Kingdom and Germany, with a background of MRSA infections [26-29]. The emergence of VRSA/VISA was assumed to be due to building of selective pressure of vancomycin [26]. Thus, we assume that the mere use of this drug led to such absence in our study. ESBLs are found in a variety of pathogenic microorganisms like *Enterobacteriaceae* and *Pseudomonas aerugenosa* [30]. In India, Mohanty and his colleagues showed a very high rate of ESBL production (*Escherichia coli*: 72.31%; *Enterobacter spp*: 51.28%) [30]. In Bangladesh, a study by Rahman *et al*., (2004), at urban hospital in Dhaka, showed 43.21% *E. coli* and 39.5% *Klebsiella spp*. as ESBL producers [31]. Compared to these studies, we found comparatively less number of ESBL producing organisms but still it remains a threat for us as these enzymes are responsible for creating resistance against many classes of antibiotics, ultimately resulting in treatment failures [32].

Finally, most of the pathogenic microorganisms isolated from this study showed resistance against two or more of the commonly used antibiotics. In developing countries like Bangladesh where appropriate resources are not available to formulate new drugs, such a situation creates difficulties in treating patients with multi drug resistance (MDR). To overcome this fatal problem, we suggest random documentation of antibiotic susceptibility test for the rational and effective use of antimicrobial agents. As we accomplished, resolving the antibiotic resistance level of the important pathogens collected from clinical specimens in the Dhaka area would be important information to bring to the attention not only of the local medical community, but also for the other developing countries having the practice of antibiotic abuse [15, 24, 33-34]. Besides, using the resistance data revealed from our study, the model we proposed for the antibiotic resistance index is the first time attempt in Bangladesh, which could serve as a guideline for physicians for picking the effective antibiotic for appropriate medication in a community.

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Table I: Distribution of clinically significant pathogens.

Organisms	Urine	Pus	Wound swab	Sputum	Blood	Conjunct-ival swab	Throat swab	HVS	Stool	Total number
Escherichia coli	424 (56.46%)	06 (5.08%)	09 (16.67%)	20 (40%)	11 (31.43%)	0	0	05 (45.46%)	0	475 (45.50%)
Klebsiella spp.	98 (13.04%)	03 (2.54%)	03 (5.55%)	10 (20%)	03 (8.57%)	0	0	03 (27.27%)	0	120 (11.50%)
Staphylococcus aureus	29 (3.86%)	54 (45.76%)	17 (31.5%)	0	0	03 (27.27%)	0	0	0	103 (9.86%)
Pseudomonas spp.	12 (1.60%)	23 (19.5%)	10 (18.51%)	0	0	0	0	0	0	45 (4.31%)
Enterococcus spp.	37 (4.93%)	03 (2.54%)	05 (9.26%)	0	0	0	0	0	0	45 (4.31%)
Enterobacter spp.	16 (2.13%)	04 (3.39%)	0	09 (18%)	09 (25.71%)	0	0	03 (27.27%)	0	41 (3.93%)
Streptococcus agalactiae	37 (4.93%)	02 (1.69%)	0	0	0	0	0	0	0	39 (3.73%)
Citrobacter spp.	12 (1.60%)	01 (0.85%)	01 (1.85%)	09 (18%)	09 (25.71%)	0	0	0	0	32 (3.06%)
Beta haemolytic *Streptococci*	25 (3.32%)	02 (1.69%)	04 (7.41%)	0	0	0	0	0	0	31 (2.97%)
Staphylococcus epidermidis	02 (0.27%)	13 (11.02%)	03 (5.55%)	0	0	08 (72.73%)	0	0	0	26 (2.50%)
Proteus spp.	15 (2%)	03 (2.54%)	02 (3.70%)	0	0	0	0	0	0	20 (1.91%)
Streptococcus pyogens	08 (1.06%)	0	0	0	0	0	11 (100%)	0	0	19 (1.82%)
Acinetobacter spp.	10 (1.33%)	4 (3.39%)	0	1 (2%)	1 (2.86%)	0	0	0	0	16 (1.53%)
Candida spp.	15 (2%)	0	0	0	0	0	0	0	0	15 (1.44%)
Group D Non-*Enterococcus*	4 (0.53%)	0	0	0	0	0	0	0	0	4 (0.38%)
Streptococcus (other)	4 (0.53%)	0	0	0	0	0	0	0	0	4 (0.38%)
Moraxella spp.	3 (0.40%)	0	0	0	0	0	0	0	0	3 (0.29%)
Shigella spp.	0	0	0	0	0	0	0	0	3 (100%)	3 (0.29%)
Streptococcus pneumoniae	0	0	0	1 (2%)	0	0	0	0	0	1 (0.096%)
Salmonella typhi	0	0	0	0	1 (2.86%)	0	0	0	0	1 (0.096%)
Salmonella spp.	0	0	0	0	1 (2.86%)	0	0	0	0	1 (0.096%)
Number of isolates per specimen	751 (71.93%)	118 (11.30%)	54 (5.20%)	50 (4.78%)	35 (3.35%)	11 (1.05%)	11 (1.05%)	11 (1.05%)	03 (0.29%)	1044

Table II: Antibiotic resistance patterns exhibited by Gram negative bacteria

Organisms	AMC	CAZ	CXM	CL	CIP	TET	COT	NIT	IMI	AK	GEN	CAR	ESBL
E. coli n=475	62%	59%	58%	59%	89%	69%	64%	15%	00%	11%	26%	ND	35%
Klebsiella spp. n=120	58%	51%	52%	51%	78%	53%	49%	17%	00%	9%	11%	ND	22%
Pseudomonas spp. n=45	97%	95%	96%	96%	89%	95%	97%	100%	11%	70%	73%	94%	ND
Enterobacter spp. n=41	90%	87%	80%	87%	73%	70%	68%	12%	00%	17%	24%	ND	21%
Citrobacter spp. n=32	68%	56%	59%	59%	81%	71%	78%	14%	00%	15%	25%	ND	19%
Proteus spp. n=20	80%	85%	90%	90%	75%	90%	80%	ND	02%	5%	10%	ND	20%
Acinetobacter spp. n=16	62%	62%	62%	62%	75%	68%	75%	80%	00%	00%	43%	ND	ND

AMC: Amoxyclav (30 µg) CAZ: Ceftazidime (30 µg) IMI: Imipenem (10 µg)
CXM: Cefuroxime (30 µg) CL: Cephalexin (30 µg) AK: Amikacin (10 µg)
CIP: Ciproflaxacin (5 µg) TET: Tetracycline (30 µg) GEN: Gentamicin (10 µg)
COT: Cotrimoxazole (25 µg) NIT: Nitrofurantoin (300 µg) CAR: Carbenecillin (100 µg)
ESBL: Extended Spectrum β- Lactamase ND: Not Done

Table III: Antibiotic resistance patterns of Gram positive bacteria

Organisms	AMP	AMC	PEN	OXA	CL	CIP	TET	COT	AK	GEN	VAN	MET resistance
Staphylococcus aureus n=103	80%	66%	93%	46%	51%	69%	44%	49%	29%	31%	00%	46% MRSA
Staphylococcus epidermidis n=26	84%	76%	92%	30%	61%	73%	57%	69%	23%	34%	00%	30% MRSE
Streptococcus agalactiae n=39	97%	94%	97%	ND	56%	74%	69%	100%	33%	20%	00%	ND
Beta haemolytic *Streptococc*i n=31	96%	93%	96%	ND	61%	74%	64%	100%	58%	32%	00%	ND
Streptococcus pyogenes n=19	00%	00%	00%	00%	00%	73%	31%	100%	52%	5%	00%	ND
Enterococcus spp. n=45	13%	8%	13%	ND	22%	66%	60%	100%	62%	8%	00%	ND

AMP: Ampicillin (10 µg) AMC: Amoxyclav (30 µg) `AK: Amikacin (30 µg)
PEN: Penicillin (10 µg) OXA: Oxacillin (1 µg) GEN: Gentamicin (10 µg)
CL: Cephalexin (30 µg) CIP: Ciprofloxacin (5 µg) VAN: Vancomycin (30 µg)
TET: Tetracycline (30 µg) COT: Cotrimoxazole (25 µg) MET: Methicillin
MRSA: Methicillin Resistant *Staphylococcus aureus*
MRSE: Methicillin Resistant *Staphylococcus epidermidis* ND: Not Done

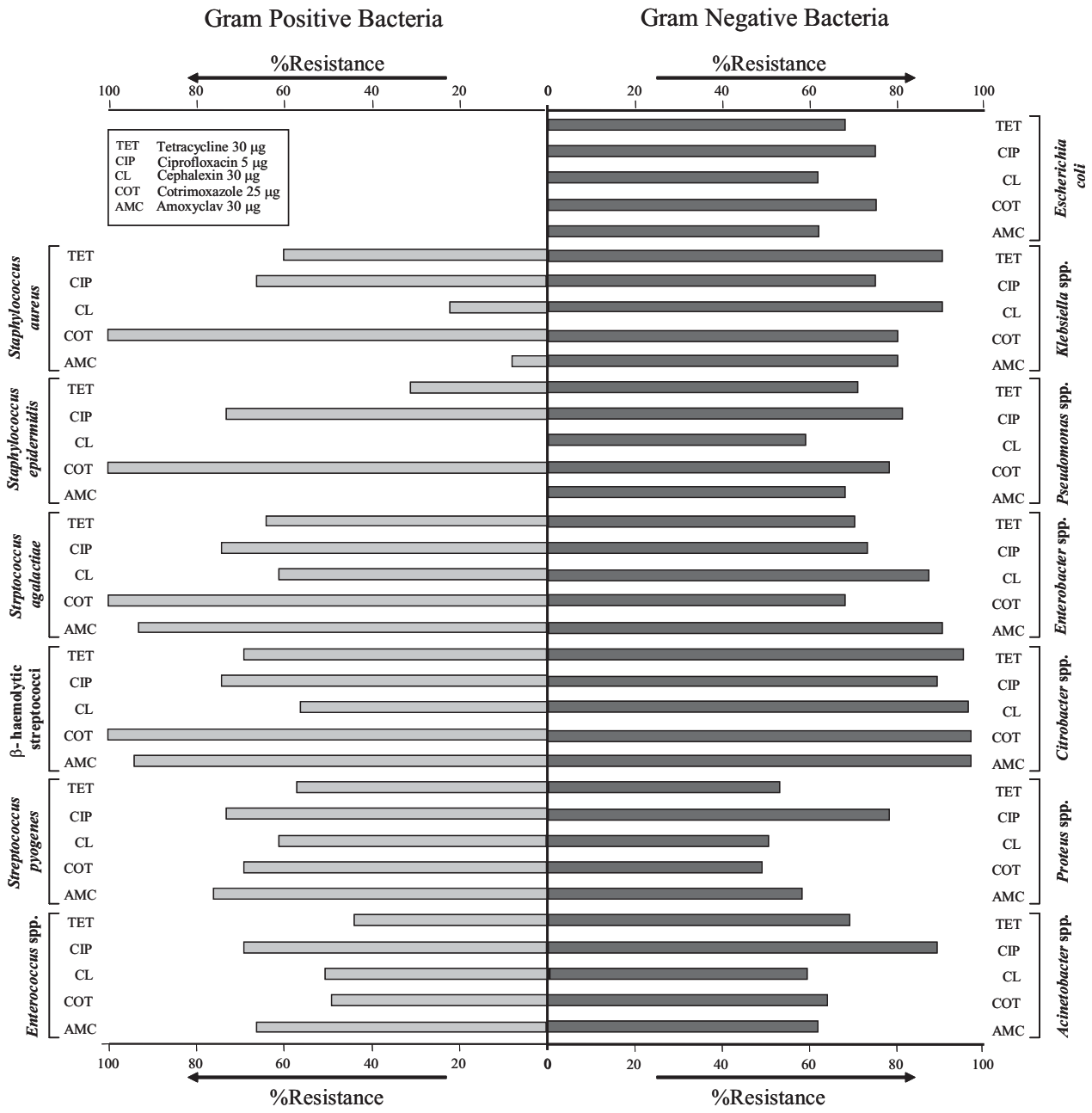

Figure I. Model of antibiotic resistance index. The resistance patterns exhibited by Gram positive and Gram negative bacteria against the similar antibiotics (amoxyclav, ciprofloxacin, tetracycline, cotrimoxazole and cephalexin) were assembled. Purple bars are indicative of resistance against Gram positive bacteria while the red bars denote the antibiotic resistance against Gram negative bacteria.

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