

Multidrug Resistance for Some Entero-bacterial Cases from Al-Yarmouk Hospital

Mariam Hamdi Abdulkareem* and Noor A. Mohammed

College of Veterinary Medicine, University of Baghdad, Iraq

Abstract

Many resistant strains had been generated due to the intensive usage for the antibiotics either by human beings or veterinary affairs. Antimicrobial susceptibility testing for isolates obtained from patients' samples revealed multidrug resistance profiles. Almost all isolated bacterial samples (nearly 90%) revealed high resistance. current study aimed for the investigation of the susceptibility rates of isolated bacterial species which are: *E coli* (60 samples), *Klebsiella* (27 samples), *Salmonella typhi* (1 sample) *Serracia marcescens* (4 samples), *Enterobacter Cloacae* (8 samples) and (*Morganella morgani*) in Al-Yarmouk hospital located in Baghdad (IRAQ). Clinical patient's specimens were gotten from various biological sources followed by the biochemical testing to be diagnosed and identified correctly, then the use of VITEC-2 system software occurred. Using the antibiotics of: Tricarillin , clavulanic acid, piperacillin , tamzbacta, ceftazidime, cefepime, aztreonam, imipenem, meropenem, amikacin, gentamicin, tobramycinhis bacteria, ciprofloxacin, minocyclin I3, trimethoprim/sulfamethoxazole; showed that there was a higher incidence of multidrug-resistance for all the tested bacterial strains; that *E.coli* showed 96% resistance against imipenem antibiotic making it the most ineffective drug against it; while *Klebsiella* bacteria showed 100% resistance against clavulanic acid and piperacillin antibiotics. When *Serracia marcescens* and *Enterobacter cloacae* showed resistance towards almost all the antibiotics used at this study. For *Morganella morgani* and *Salmonella typhi*, they showed resistance to some of the used antibiotics and were sensitive to the others. The detection of microorganisms at this high resistance to wide range of antibiotics put us in front of serious problem in present time. Thus, clinicians might benefit from these data to enhance the usage of antibiotics and follow moderate protocol to minimize the hazards.

Keywords: antimicrobial resistance; multidrug resistance, hospital isolates, Al-Yarmouk hospital.

1. Introduction

Resistance of microbes toward antibiotics has been increased markedly at the atmosphere naturally according to the frequent usage of medications either in hospitals or in the entire community, parallel to their major use in animal husbandry and veterinary sponsor [1]. Some bacterial pathogens proved their elevated level of resistancy towards huge number of drugs including the group that described as: ESKAPE for (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.); they were from the initial line and final hope antibiotics. In spite of that, clinical atmosphere was always a rich area to grow these bacterial species that it is responsible of plenty of hospital-acquired infections; however ESKAPE members were gotten from different sites as well, such as food, water surface, soil and waste-water [2]. The problem may risen due to the hospital sewage littering into the natural environments and this would facilitate bacterial strains spread leading to enhance the virulence mechanisms causing an uncontrolled distribution, beside spreading of mobile genetic elements which carry genes that encoding for resistance accompanied by the virulence genes; especially the areas that exposed to hospital discharges in their surroundings [3] and Al-Yarmouk hospital is located inside the capital of Iraq: Baghdad.

Unfortunately, using broad-spectrum antibiotics such as aminoglycosides, thirdand fourth-generations fluoroquinolones and cephalosporins, led multidrug-resistant bacterial isolates to be opportunistic; Hence, it can colonize within the patients and may result in major infections untreatable at some

cases [4,5]. In both humans and animals, their intestine has the capability to harbor bacteria and produce infection in hospitals as well as community [6]. Naturally, selecting bacterial strains that are multidrug-resistible might take place in natural environment through producing some compounds with antibiotic features in soil and through waste of human or feces of animals as well, which possibly might carry residues of antibacteril drugs [7,8]. Rottenly, some genes that encoding for resistance might be by chromosome housekeeping genes which are responsible of bacterial auto-protective mechanisms [9]. Besides, few antibiotics are added at the farms of the animals and the aquaculture to promote growth and to protect the culture (prophylactic agents) resulting in the foundation of artificial bacterial resistancy selection [10]. Using bacterial inhibitory drugs for mankind or even in veterinary might facilitate selecting strains of bacteria with abnormal resistance causing by that strengthen the efficiency of these strains for infecting, producing by this a new developed generation with elevated power of resistance [11,12].

On other hand, there is a serious problem by the presence of mobile genetic elements at few bacterial strains as transposons and plasmids, those might horizontally to the next generations creating by this an encode to resisting mechanisms against several antimicrobial drugs [13,14]. Thus, ecosystem was influenced directly by the wastes [15].

Al-Yarmouk Hospital located in Baghdad/ Iraq; it's a huge hospital which suffered from intense degradation along its distances because of the growth of the population and the development of the industry [16], hence, one of the most effecting outcomes was the hospitals wastes and sewages in the hospitals area. The purpose of our work was to diagnose and identify enteric bacteria which isolation was done through cases visited Al-Yarmouk hospital.

2. Materials and methods

2.1. Samples collection, culturing and identification from clinical materials

Samples collection was from patients visited Al-Yarmouk Hospital at Baghdad city; isolation done from throat as swabs, sputum, feces or biopsies. bacterial samples isolation were about 101, which were transported with transported media to the laboratory inside Al-Yarmouk hospital from September 2022 to January 2023 that different culture media were used for the bacterial isolation and identification including (basically MacConkey agar) as following: *E coli* (60 samples), *Klebsiella* (27 samples), *Salmonella typhi* (1 sample) *Serracia marcescens* (4 samples), *Enterobacter Cloacae* (8 samples) and *Morganella morgani* (1 sample); then incubated under aerobic conditions at 37°C for 24-48 hrs., gram staining and colonies characteristic features were studied (shape, color, size, consistency, and production of pigments), this was done according to Quinn & Forbes [17,18]. Many biochemical tests were used beside VITEK-2 system.

In order to identify isolated bacteria, there was a need to use of coagulase, catalase, oxidase, urease, gelatin liquefaction, hemolysis on blood agar, carbohydrate utilization and motility tests, IMViC and TSI, then bacteria were diagnosed followed by preparing to susceptibility test.

2.2. Antimicrobial susceptibility tests

Kirby–Bauer disk diffusion assay was in use (on Muller- Hinton agar plates) in order to examine bacterial susceptibility, that bacteria cultured on test plates followed by inserting the disks which contain antibiotic onto the lawn of bacteria and incubated, to read the zone of inhibition and measure it; however, the isolates were mentioned as: resistant (R), intermediate (I), susceptible (S) according to CLSI guidelines [19]. Followed by using these antibiotic disks with their potency on them: Tricarillin , clavulanic acid (10 µg), Piperacillin , Tamzbacta (10µg), ceftazidime (30µg), cefepime (30µg), aztreonam (Atm30µg), imipenem (lpm- 10µg), Meropenem , amikacin (Ak- 30µg), gentamicin (Gen-10µg), tobramycin (Tob-10µg), ciprofloxacin (Cip-10µg), Minocyclin I3 , Trimethoprim/ sulfamethoxazole.

Nephelometer was used via suspension with turbidity similar to that of McFarland tube no. 0.5 as a standard to achieve by emulsifying the test bacterial isolates in normal saline; followed by streaking bacterial suspension evenly on the surface of the plate of Mueller Hinton agar to ensure obtaining of semi-confluent growth after incubation. All plates were incubated at the same incubation circumstances (37°C for 24hours) followed by measuring the zones of inhibition around the disks containing antibiotics.

3.Results and Discussion

Results were listed as showing in the table 3.1

Table 3.1 Bacterial resistance and sensitivity towards antibiotics

	<i>E coli</i>	<i>Klebsiella</i>	<i>Serracia marcescens</i>	<i>Enterobacter Cloacae</i>	<i>Morganella morgani</i>	<i>Salmonella typhi</i>
Samples no.	60	27	4	8	1	1
Tricarillin	R=46 S=14	25 5	3 1	8 -	- 1	1 -
Clavulanic acid	R=35 S=25	27 -	4 -	6 2	- 1	1 -
Piperacillin	R=25 S=10	27 -	- -	8 -	- 1	1 -
Tamzacta	R=24 S=16	25 2	3 1	1 7	- 1	- 1
Ceftazidine	R=21 S=39	24 3	- -	2 6	- 1	1 -
Cefepime	R=19 S=9	22 4	4 -	- 8	- 1	1 -
Azteonam	R=34 S=26	19 8	1 3	8 -	- 1	1 -
Imipenem	R=58 S=2	20 7	- -	- 8	- -	- 1
Meropenem	R=59 S=1	22 5	4 -	- 8	- 1	- 1
Amikacin	R=44 S=16	19 8	3 1	2 6	- 1	- 1
Gentamicin	R=24 S=36	12 15	4 -	2 6	1 -	- 1
Tobramycin	R=28 S=32	- -	4 -	1 7	1 -	- 1
Ciproflaxicin	R=14 S=8	- -	1 3	2 6	- 1	1 -
Minocyclin I3	R=26 S=31	7 20	2 1	- 8	1 -	- 1
Trimethoprim/ sulfamethoxazole	R=29 S=10	18 9	2 2	- -	1 -	- 1

- resistant (R), intermediate (I), susceptible (S)

3.1. Reading antimicrobial susceptibility test and ESBL confirmation test

Determining multidrug-resistant profiles for the bacterial strains was done using various antimicrobial drugs according to the base of those antibiotics which first to be expected efficient for therapy until those less in use in the medication. In samples isolates for *Escherichia coli*, sixty samples were isolated, shown in figure (1), 46 (77%) of them were resistant (R) to Tricarillin antibiotic and 14 (23%) revealed sensitivity (S) to the same antibiotic. At the time that Clavulanic acid revealed 35 (58%) of the samples were R and 25 (42%) samples were S. When using Piperacillin, 25 samples (42%) were resistant to the antibiotic while when we used Tamzbacta, 24 samples (40%) were resistant. For the Ceftazidime, 21 samples (35%) were resistant. And fro Cefepime, resistance showed by 19 samples (31%). When using Azteonam, 34 samples (56%) were resistant to this antibiotic. Furthermore, Imipenem antibiotic revealed 58 samples (96%) which were resistant. Also Meropenem gave 59 resistant samples (98%). Amikacin antibiotic revealed 44 resistant samples (73%). While Gentamicin showed 24 resistant samples (40%); and Tobramycin revealed 28 resistant behaviors (46%); but Ciproflaxicin showed 14 resistant samples only (23%), when Minocyclin I3 showed 26 resistant samples (43%); finally, Trimethoprim/sulfamethoxazole mixture revealed 29 resistant samples (48%).



Figure (1) *E.coli* bacteria isolated at the laboratory of AL-Yarmouk Hospital

Klebsiella bacterium was isolated at the rate of 27 samples revealing resistance 25 (92%) to Tricarillin antibiotic and 5 (18%) revealed sensitivity (S) to the same antibiotic. At the time that Clavulanic acid and Piperacillin revealed 27 (100%) of the samples were resistant to these antibiotics; while when we used Tamzbacta, 25 samples (92%) were resistant. For the Ceftazidime, 24 samples (88%) were resistant. And for Cefepime and Meropenem antibiotics, resistance was 22 samples (81%). When using Azteonam or Amikacin antibiotics, 19 samples (70%) were resistant. Furthermore, Imipenem antibiotic revealed 20 samples (74%) which were resistant. While Gentamicin showed 12 resistant samples (44%) and 15 (56%) sensitive; when Minocyclin I3 showed 7 resistant samples (25%) and 20 sensitive (75%); finally, Trimethoprim/sulfamethoxazole mixture revealed 18 resistant samples (66%).

Subsequently, for the *Serracia marcescens*, the samples isolated were 4, mostly all the antibiotics used, the bacteria was resistant to them at the percentage of 75 or 100% except for the antibiotics: Azteonam, Ciproflaxicin in which the bacterium was sensitive to them, and finally it was intermediate to the antibiotics mixture Trimethoprim/ sulfamethoxazole.

Enterobacter cloacae bacterium (8 isolated samples) showed another trend at its behavior towards the drugs that it was resistant to Tricarillin antibiotic, Clavulanic acid, Piperacillin and Azteonam at about 75-100%; On the contrary, when Tamzbacta, Ceftazidime, Cefepime, Amikacin, Gentamicin the bacteria was sensitive to them at a percentage ranging from 75-87%; moreover, *Enterobacter cloacae* was sensitive 100% to Cefepime, Imipenem, Meropenem and Minocyclin antibiotics.

Additionally, *Morganella morgani* bacterium showed different attitude when only one sample isolated at the laboratory that it was resistant to Gentamicin, Tobramycin, Minocyclin I3 and Trimethoprim/sulfamethoxazole 100%; and it was sensitive to Tricarillin, Clavulanic acid, Piperacillin, Tamzbacta, Ceftazidone, Cefepime, Azteonam, Meropenem, Amikacin and Ciproflaxacin

Similarly, *Salmonella typhi* was also isolated from one patient only, revealing resistance to Tricarillin, Clavulanic acid, Piperacillin, Ceftazidone, Cefepime and Azteonam 100%; while sensitivity was revealed towards Tamzbacta, Imipenem, Meropenem, Amikacin, Gentamicin, Tobramycin, Minocyclin I3 and Trimethoprim/sulfamethoxazole.

In spite of applying strong antimicrobial drugs against hospital isolates, there were still, at least, one bacterial isolate to reveal resistance. Rare differences at the resistance profiles were noticed between *Morganella morgani* and *Salmonella typhi* isolates using different antimicrobial classes.

4. Discussion

Multidrug-resistant strains might arise at areas with high impact population due to littering from hospital community through discharges to the colonized humans [20] and contacting with patients through nosocomial infections; leading to spread of bacterial agents [21]; thus causing usage of antibiotics between these systems, raising the intensive use of drugs among populations, leading to activating bacterial agents to stimulate their alteration to survive termination through mutations and resistance mechanisms. It was obvious that *E.coli* showed resistance against Meropenem gave (98%) and its sensitivity exhibited towards Gentamycin (40%); few researchers assumed that specific genes in Enterobacteriaceae could be encoding to resistance mechanisms for beta-lactamases and quinolone carried on the same plasmid made them transferors to other species [22].

Polymerase chain reaction techniques confirmed the occurrence of resistance through few genes to anti-microbial group agents or classes for Enterobacteriaceae strains isolated from varied sources associated to human infections [23], reports proved that there are integrons containing genes which encode resistance to Extended-spectrum beta-lactamases (ESBLs) (enzymes applies resistance to many beta-lactam antibiotics such as penicillins, cephalosporins, and the monobactam aztreonam.) so that any infection with ESBL-producer organisms could reveal high resistant bacterial agent against the used antibiotic, these ESBLs are available in *E.coli*, *Klebsiella nemoniae*; hence, providing high level of resistance, they have genes encoding for ESBLs beside plasmid-mediated quinolone resistance related to integrons and contained in plasmids to be passed via conjugation [24] in addition to the resistance towards to aminoglycosides (including gentamycin). According to Magiorakos and his co-workers [25], gentamycin resistance could be an indicator for multidrug-resistant profile. Likewise, When noticing the table (1) for the genus *Klebsiella*, it has the highest resistant towards Clavulanic acid and Piperacillin that (100%) of the samples were resistant to these antibiotics; at the same time, sensitivity percentage was (75%) towards Gentamycin, may the cause be attributed to some mobile genetic elements (transposons or insertion sequences) are widely spread at natural environment, water, soil, and hospital settings micros; providing by this resistance for the microbe against antibiotics, especially when we knew that these genes were similar to those available in pathogenic bacterial isolates [26]. Furthermore, The bacterium *Serracia marcescensis* had resistance towards Clavulanic acid, Cefepime, Meropenem, Gentamicin and Tobramycin at the percentage of 100% except and most of its sensitivity was to: Azteonam and Ciproflaxacin (75%); Many elements are responsible of the resistance mechanism towards the used antibiotic, such as the resistance to imipenem and Meropenem, microbes exposed to fourth generation Cephalosporin's and Carbapenems seems to have mechanism facilitate the changing in cell wall permeability by minimizing of gene expression for cell-wall proteins of the membrane expressing by this the resistance to meropenem [27]. Additionally, *Enterobacter cloacae* bacterium showed resistant to Tricarillin antibiotic, Clavulanic acid, Piperacillin and Azteonam at about 75-100, moreover, *Enterobacter cloacae* was sensitive 100% to Cefepime, Imipenem, Meropenem and Minocyclin

antibiotics. Parallel to the findings of a researcher [28] who mentioned that there is variability in the resistance behavior of *Enterobacter* spp towards different antibiotics such as ceftriaxone, aztreonam, ceftazidimeclavulanic acid, cefepime, ceftazidime, cefotaxime.

Since the information are outlined about the ESKAPE pathogens, (*Enterobacter* included) antibiotic resistance still a mystery to the scientists, though they tried to expose it to the artificial intelligence hoping that they find the beginning of the thread [2]. However, few antibiotics from the cephalosporins (such as cefepime, ceftazidime) and from carbapenems (such as meropenem, ertapenem, doripenem) are useful and effective against the infections of *Enterobacter* spp. [29]. Furthermore, aminoglycosides such as amikacin, revealed major effectivity in treating more than 95% of *Enterobacter* spp. infections [30].

Morganella morganii the species that considered an unusual opportunistic pathogen which mainly is the causative agent of infections in wound after operations as well as in urinary tract; these infections might represents major issue in clinical infectious controlling because of the present of multiple number of antibiotics resistance through multiple genes in certain clinical isolates leading to high mortality rate in patients with some infections [31]; This bacterium might provide extended-spectrum β -lactamases & carbapenemases, thus increasing resistance to multiple antibiotics leading to elevated mortality range that many reporters recommended an effective treatments to control this very bacterium multidrug-resistance [32]. Generally, *Morganella* infection resulted from either multidrug-resistant or by extensively drug-resistant leading to treatment failure, because of its capability of producing virulence factors, as examples: hemolysins, urease or lipopolysaccharide (LPS) making it an opportunistic pathogen with analysis of its genome revealed few pathogenicity related genes beside some novel providing precious information concerning virulent genes in *M. morganii* [33]. A research held in Al Najaf hospital in Iraq [34], the researcher mentioned that *Morganella morganii* may cause outbreaks of septicemia and bacteremia, especially with patients post-surgery because of the beta-lactam antibiotic resistance which became ordinary. Meropenem and imipenem antibiotics were noticed to be efficient towards *M. morganii* bacteria. Moreover, shifting at the use of medications to treat animals such as treating the infections of *Salmonella* in poultry from antibiotic to another caused intensive resistance to most of the regularly used antibiotics [35].

5. Conclusion

Entire isolates revealed resistance towards most in use antimicrobials, leading to the limitation for options of treatment. This study may provide useful sparkles to produce new antibiotics based on genetic targets against infectious agents. However, future studies could be performed in a comparative pathway to isolate and identify other resistant strains in other hospitals.

6. References

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