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Isolation and Laboratory Diagnosis of Antibiotic-resistant *E. coli* from Surgical Wounds of Inpatients at Zagazig University Hospitals, Egypt

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Abstract *E. coli* is one of the most common community and nosocomial pathogens and it mainly causes skin and soft tissue infections. So, the current study aimed to study the isolation of antibiotic-resistant *E.coli* strains in Egypt, Hospitals. Study their antibiotic susceptibility profile and therapy to control and present the transmission of *E. coli* strains among the health care settings.120 pus samples were collected from post-operation wounds of a study group of inpatients in Egypt, Hospitals. Bacteria colonizing the wounds were isolated on specific culture media thereafter purified and divided into 4 groups depending on their morphological characters and Gram's stain reaction Isolated bacteria were divided into 4 groups. Group (I) represented 40% of total isolates; while group (II) & (III) represented 25% and group (IV) 22%. Isolated bacteria related to groups I, II, III & IV were preliminary identified as *S. aureus. E. coli, K. pneumoniae* and *P. aeruginosa,* respectively. Antibiotic susceptibility of the 4 groups of isolated bacteria against 10 different antibiotics revealed that Ciprofloxacin showed the highest activity against *E. coli.* Data revealed that strain encoded *E.coli 6* is a multi-resistant strain as it resisted 13 antibiotics out of 15 (86.7%).

Keywords: *E. coli, nosocomial pathogens, wounds, ciprofloxacin, vancomycin*

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1. Introduction

Most bacteria live on our skin, in the nasopharynx, gastrointestinal tract and other parts of the body with little potential for causing disease because of first-line defense within the body. The surgical operation, trauma, burns, diseases, nutrition and other factors affect these defenses. The skin barrier is disrupted by every skin incision, and microbial contamination is inevitable despite the best skin preparation [1]. The primary cause of antibiotic resistance is a genetic mutation in bacteria. Also, the prevalence of antibiotic-resistant *E. coli* is a result of antibiotic use both within medicine and veterinary medicine [1]. The contaminating pathogens in gastrointestinal surgery are the multitude of intrinsic bowel flora, which include Gram-negative bacilli (e.g *Escherichia coli)* Antibiotic resistance genes can be horizontally transferred between (between individuals) by conjugation, transduction or transformation. If a bacterium carries several resistance genes, it is called multiresistant or, informally, a superbug or super bacterium [2,3]. The glycopeptide antibiotic vancomycin was introduced clinically in 1958 for the treatment of gram-positive bacteria. Use of this agent has increased dramatically in the last 20 years, in large part because of the increasing prevalence of methicillin resistance *E. coli* [4].

Among aerobic Gram-negative bacteria, *Escherichia coli* was the most predominantly isolated from primary infections followed by *Klebsiella pneumoniae* and *Pseudomonas aeruginosa,* in this order, and from postoperative infections, *P. aeruginosa* was most predominantly isolated, followed by *E. coli, Enterobacter cloacae, and K. pneumoniae.* The emerging threat of widespread vancomycin resistance possess a serious public health concern given the fact that vancomycin has long been the preferred treatment of antibiotic-resistant gram-positive organisms [4]. So the current study aimed to study isolation of antibiotic-resistant *E. coli* strains in Egypt, Hospitals**.**

2. Materials and Methods

1. Sample collection.

120 medical specimens of pus were collected from patients in different Departments of Egypt Hospitals.

2- Isolation and purification of bacterial isolates.

2.1-Isolation:

Samples were collected from wounds infections as pus. Samples were handled by the sterile swab. All collected

samples were transferred to the laboratory within 2-3 hours and streaked on nutrient agar to obtain single colony.

2.2-Purification.

The swabs were streaked on agar surface of different diagnostic and selective media namely, (C.L.E.D agar), Nutrient agar, MacConkey agar, and Blood agar and Uri select media.

Plates were incubated aerobically at 37˚C for 24 h. Growing colonies were purified and examined for their systematic position using cultural characters and Gram's stain preparation.

3- Identification of bacterial strains:

The isolated bacterial strains were identified by the following tests:

Nitrate reduction, Motility test, Arginine hydrolysis, Gelatin liquefaction, Oxidase test, Citrate Utilization, Methyl red, Triple sugar iron agar, Catalase activity, Coagulase test.

4- Antibiotic susceptibility test.

4.1- Antibiotic disks:

Ten antibiotics were selected for carrying out the antimicrobial susceptibility test. Imipenem (IPM), Vancomycin (VAN), Chloromphenicol (C), Cefotaxime (CTX), Cefepime (FEP), Cephardin (CE), Amoxycillin (AX), Ampicillin \ sulbactam (unasyn) (SAM), Ampicillin (AM), Ciprofloxacin (CIP).The antibiotic disks used in this research were purchased from Oxoid Ltd., England.

4.2- Disk diffusion agar method:

Antibiotic susceptibility test for the bacterial isolates was carried out by disk diffusion technique according to [5].

5. Determination of the Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations(MBCs) of different antibiotic against *E.coli* isolates.

Not all the above-mentioned antibiotics were tested by this method against different strains including *E.coli*, only one antibiotic was used, according to the activity against the tested bacterial organism. Ciprofloxacin (CIP), for *E.coli*, for purchased from Oxoid Comp. MICs and MBCs were determined by using the standard broth dilution technique [6].

3. Results

1. Isolation of bacteria colonizing post-operation wounds of inpatients at Zagazig University Hospitals

120 pus samples were collected from post-operation wounds of inpatients in different departments at Egypt, Hospitals. The study group included 70 males and 50 females. Bacteria colonizing the wounds were isolated on specific culture media thereafter purified and divided into 4 groups depending on their morphological characters and Gram's stain reaction.

The 4 groups included Gram +ve Staphylococci (I). Gram -ve non motile rods growing on ordinary media(II). Gram -ve non motile rods growing on ordinary and MacConkey media(III). Gram-ve motile rods growing on ordinary and MacConkey media (IV). Bacteria related to Group I represented 40% of total isolates: while group (II) & (III) represented 25% and group (IV) 22%.

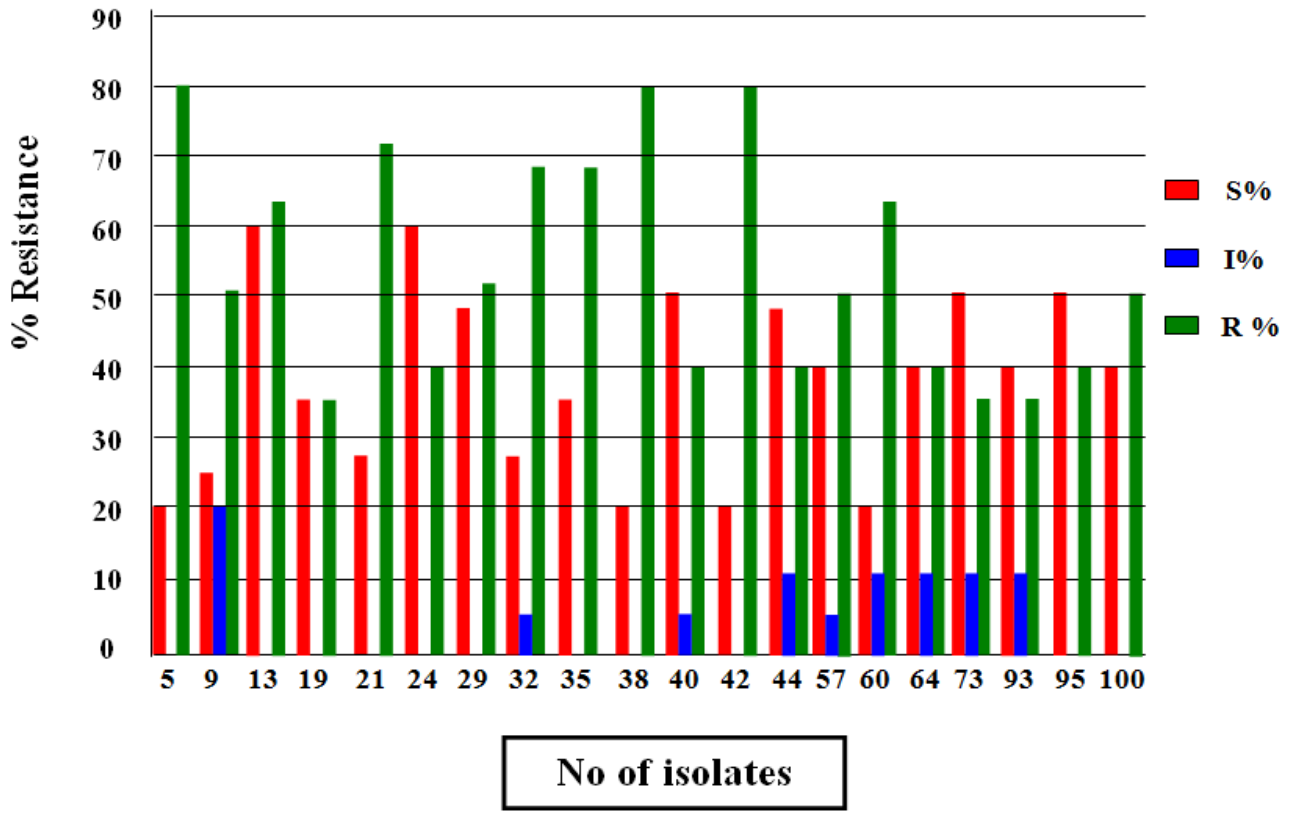

Figure 1. Resistance % of *E.coli* strains to (10) tested antibiotics

2. Antibiotic susceptibility of the 4 groups of bacteria

Table 1. Determination of minimum inhibitory concentration (MICs) and minimum bactericidal concentration (MICs) of Ciprofloxacin antibiotic against selected multi-resistant strains

no	Isolate	MIC (µg/ml)	MBC (µg/ml)
1	*E. Coli* 6	32.45	67.5
2	*K. pneumonia* 44	32.45	67.5
3	*P. aeruginosa* 39	62.5	125
4	*S. aureus 7*	62.5	62.5
5	*S. aureus 27*	32.45	67.5

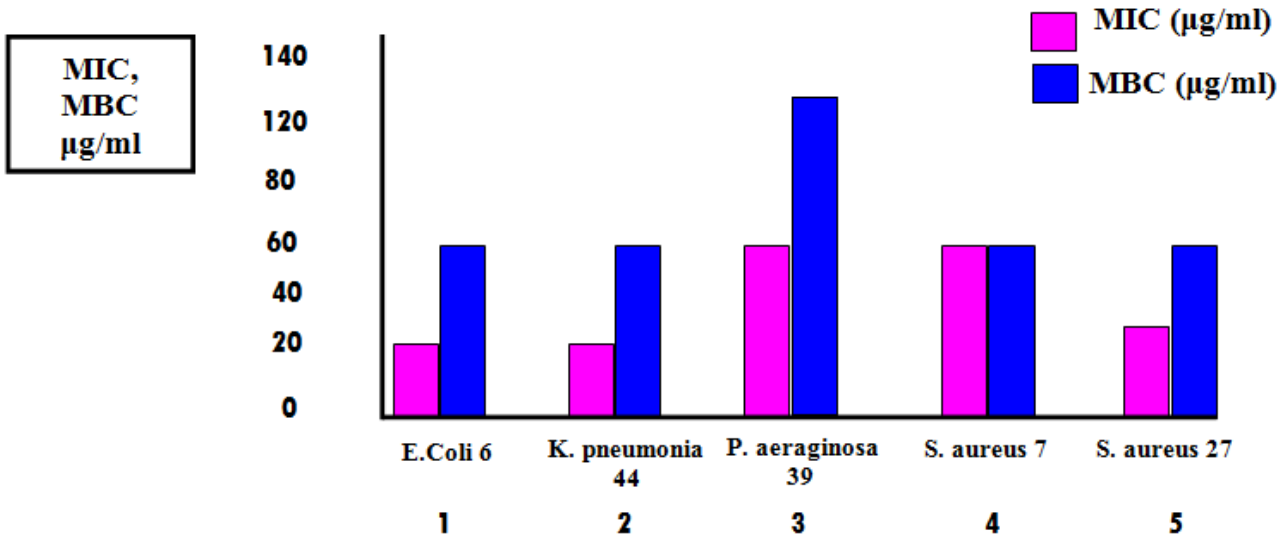

Figure 2. MICs and MBCs of Ciprofloxacin antibiotic against multi-resistant strains

4. Discussion

Bacterial agents often incriminated with wound infections include *Staphylcoccus*, *Klebsiella*, *E. coli* and *Proteus* as well as anaerobes as *Clostridium* and *Bacteriodes* species the most common group of bacteria responsible for SSIsis *Stuphyloccusaureus.* [7].

The present study was initiated by a collection of 120 specimens of pus from wounds of inpatients in different departments Egypt, Hospitals. Bacterial isolates were purified and initially identified depending on morphologic characteristics and Gram's stain reaction. They were divided into 4 main groups; group I included 40 Gram-positive bacterial isolates (40%) preliminary identified as *Staphylococcus aureus.* Groups II, III & IV included Gram-negative bacterial isolates (62%) related to *E. coli* (25%), *K. pneumonsae* (25%) and *P. aeruginosa* (22%), respectively.

Previous studies observed that though the percentage of Gram-negative bacilli from the wounds was more, *Staphylococcus aureus* was predominant organism isolated followed by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* .and *Escherichia coli***.** [8,9,10,11,12].

Another study showed that the isolation rate of 52% in which *S.aureus* was the predominant species 65% (51/79) followed by *E.coli*, 8/79 (10%), *K.pneumoniae*9% (7/79), *Proteus* species 4% (3/79) and Streptococci species 4% (3/7). The organisms most frequently involved in surgical infections change from time to time, and also vary with hospital selling. This difference may be due to variation in common nosocomial pathogens inhabitant in a different hospital set up [13].

In this study, the antibiotic susceptibility pattern of 120 bacterial isolates to 10 different antibiotics was investigated by using disc diffusion method. The included antibiotics were: imipenem (IPM), Vancomycin (VAN), Chloromphenicol (C), Cefotaxime (CTX), Caffeine (FEP), Cephardin (CE), Amoxycillin (AX), Ampicillin \ sulbactam (unasyn) (SAM), Ampicillin (AM), Ciprofloxacin (CIP).

Results revealed the varying response of bacterial isolates to the investigated antibiotics. Data showed that Gram-negative isolates collected from different source (62 isolates) were resistant to AX, CE, CTX, VAN, AM, and MET with a percentage between 45 to 85%. On the other hand, Gram-positive isolates (38%) showed a lower sensitivity to the tested antibiotics between 7.9 to 63.2.

Similarly, [9] observed very low susceptibility rate (10% sensitivity) for ampicillin and amoxicillin against all Gram-positive and Gram-negative bacteria isolated from surgical wound infections. Also [14] reported that the resistance of 94 strains of *P.aeruginosa* and *E. coli* studied to 12 β-lactam antibiotics and found that all tested isolates were resistant to at least 7 β-lactam antibiotics (amoxicillin, amoxicillin/clavulanic acid, Caffeine and Chloromphenicol

Results showed that Ciprofloxacin proved to have a broad spectrum and high activity against all the tested Gram-positive and Gram-negative bacterial isolates. [15,16].

Data revealed that strain encoded *E.coli* 6 is a multi-resistant strain as it resisted 8 antibiotics out of 10 (86.7%). Also, strains *E. coli* 38 & 42 showed resistance to 80% of all tested antibiotics. On the other hand, *E. coli* 95 was the most sensitive strain showing sensitivity to 6

antibiotics out of 10 (60%) followed by 13, 24 &73 being sensitive to5 out of 10 antibiotics (53.3%).

Enterobacteriaceae isolates resistant to multiple antibiotics have been reported from several purls in the world [17]. *P. aeruginosa* is an opportunistic pathogenic bacterium which is usually very hard to control by antibiotic therapy. Ciprofloxacin being effective against tested *E.coli* with 65.8% effectiveness was examined for its MIC and MBC.

5. Conclusion

In conclusion, the control of emergence and spread of antimicrobial resistance among the most common human bacterial pathogens is probably one of the most important challenges for the scientific and medical community. This emergence was significantly marked in hospitals that had been endemic with (MRSA) strains and followed the policy of empirical use of Ciprofloxacin and vancomycin are the most effective antibiotics for treatment of patients with serious *E. coli* infections for some decades has exerted considerable selection pressure on *E. coli* strains in the healthcare setting.

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