

AL \_ Qadisiyah University

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Isolation and Identification of *pseudomonas aeruginosa* in different clinical cases

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**اقرأ باسمِ ربِّكَ الَّذي خلَق (1) خلَقَ الإنسانَ من علَق (2) اقرأ وربُّكَ الأكرم (3) الَّذي علَّمَ بالقلَم (4) علَّم الإنسانَ ما لم يعلم (5)**

**العلق**

Summary

 This study was done to assess the bacterial profile and antibiotic sensitivity pattern of different clinical cases .

 For optimal identification of causative microbial ,where many samples are collected from different patients with different clinical cases, cultured and subjected to appropriate biochemical tests.

 These samples were collected from Laboratories in AL Qadisiyah during the study period .The antimicrobial sensitivity test was carried out by disc diffusion technique using Muller-Hinton agar ,where the results of the study showed that 20 samples gave a positive result for pseudomonas aeruginosa while 40 sample gave a negative result due to the presence of fungal pathoges.

 The majority of *pseudomonas aeruginosa* give susceptibility to the antibiotics whereas others was resistance to the antibiotics .

***Introduction:***

*Pseudomonas aeruginosa* is a common Gram-negative, rod-shaped bacterium that can cause disease in plants and animals, including humans. A species of considerable medical importance*, P. aeruginosa* is a multidrug resistant pathogen recognized for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses – hospital-acquired infections such as ventilator-associated pneumonia and various sepsis syndromes**.[1]**

The organism is considered opportunistic insofar as serious infection often occurs during existing diseases or conditions – most notably cystic fibrosis and traumatic.[1],[2]

It is also found generally in the immunocompromised but can infect the immunocompetent as in hot tub folliculitis. Treatment of P. aeruginosa infections can be difficult due to its natural resistance to antibiotics. When more advanced antibiotic drug regimens are needed adverse effects may result.[3],[2]

It is citrate, catalase, and oxidase positive. It is found in soil, water, skin flora, and most man-made environments throughout the world. It thrives not only in normal atmospheres, but also in low-oxygen atmospheres, thus has colonized many natural and artificial environments. It uses a wide range of organic material for food; in animals, its versatility enables the organism to infect damaged tissues or those with reduced immunity. The symptoms of such infections are generalized inflammation and sepsis. If such colonization occur in critical body organs, such as the lungs, the urinary tract, and kidneys, the results can be fatal. Because it thrives on moist surfaces, this bacterium is also found on and in medical equipment, including catheters, causing cross-infections in hospitals and clinics. It is also able to decompose hydrocarbons and has been used to break down tarballs and oil from oil spills. P. *aeruginosa* is not extremely virulent in comparison with other major pathogenic bacterial species – for example Staphylococcus *aureus* and Streptococcus pyogenes – though P. *aeruginosa* is capable of extensive colonization, and can aggregate into induring biofilms.[1],[2],[3]

***Cellular cooperation***

P. *aeruginosa* relies on iron as a nutrient source to grow. However, iron is not easily accessible because it is not commonly found in the environment. Iron is usually found in a largely insoluble ferric form. Furthermore, excessively high levels of iron can be toxic to P. aeruginosa. To overcome this and regulate proper intake of iron, P. aeruginosa uses siderophores, which are secreted molecules that bind and transport iron. These iron-siderophore complexes, however, are not specific. The bacterium that produced the siderophores does not necessarily receive the direct benefit of iron intake. Rather, all members of the cellular population are equally likely to access the iron-siderophore complexes. Members of the cellular population that can efficiently produce these siderophores are commonly referred to as cooperators; members that produce little to no siderophores are often referred to as cheaters. Research has shown when cooperators and cheaters are grown together, cooperators have a decrease in fitness, while cheaters have an increase in fitness. The magnitude of change in fitness increases with increasing iron limitation. With an increase in fitness, the cheaters can outcompete the cooperators; this leads to an overall decrease in fitness of the group, due to lack of sufficient siderophore production. These observations suggest that having a mix of cooperators and cheaters can reduce the virulent nature of *P. aeruginosa .[3],[4],[5]*



Pathogenesis:

An opportunistic, nosocomial pathogen of immunocompromised individuals, P. aeruginosa typically infects the airway, urinary tract, burns, and wounds, and also causes other blood infections. t is the most common cause of infections of burn injuries and of the outer ear (otitis externa), and is the most frequent colonizer of medical devices (e.g., catheters). Pseudomonas can be spread by equipment that gets contaminated and is not properly cleaned or on the hands of healthcare workers. Pseudomonas can, in rare circumstances, cause community-acquired pneumonias, as well as ventilator-associated pneumonias, being one of the most common agents isolated in several studies. Pyocyanin is a virulence factor of the bacteria and has been known to cause death in *C. elegans* by oxidative stress. However, salicylic acid can inhibit pyocyanin production. One in ten hospital-acquired infections is from Pseudomonas. Cystic fibrosis patients are also predisposed to *P. aeruginosa* infection of the lungs. *P. aeruginosa* may also be a common cause of "hot-tub rash" (dermatitis), caused by lack of proper, periodic attention to water quality. Since these bacteria like moist environments, such as hot tubs and swimming pools, they can cause skin rash or swimmer's ear. Pseudomonas is also a common cause of postoperative infection in radial keratotomy surgery patients. The organism is also associated with the skin lesion *ecthyma gangrenosum. P. aeruginosa* is frequently associated with osteomyelitis involving puncture wounds of the foot, believed to result from direct inoculation with *P. aeruginosa* via the foam padding found in tennis shoes, with diabetic patients at a higher risk.[3],[4],[5]

|  |  |  |
| --- | --- | --- |
| Infections | Details and common associations | High \_ risk groups |
| Pneumonia | Diffuse bronchopneumonia | Cystic fibrosis patients |
| Septic shock | Associated with a purple \_ black skin lesion *ecthyma* *gangrenosum*  | *neutropenic* patients |
| Urinary tract infection | Urinary tract catheterization |  |
| Gastrointestinal infection | Necrotizing *enterocolitis* | Premature infants and *neutropenic* patients |
| Skin and soft tissue infections | Hemorrhage and necrosis |  |

Toxins:

*P. aeruginosa* uses the virulence factor exotoxin A to inactivate eukaryotic elongation factor 2 via ADP-ribosylation in the host cell, much as the diphtheria toxin does. Without elongation factor 2, eukaryotic cells cannot synthesize proteins and necrotise. The release of intracellular contents induces an immunologic response in immunocompetent patients. In addition P. aeruginosa uses an exoenzyme, ExoU, which degrades the plasma membrane of eukaryotic cells, leading to lysis. Increasingly, it is becoming recognized that the iron-acquiring siderophore, pyoverdine, also functions as a toxin by removing iron from mitochondria, inflicting damage on this organelle.[5],[6],[7]

*Phenazines*

are redox-active pigments produced by P. aeruginosa. These pigments are involved in quorum sensing, virulence, and iron acquisition. P. aeruginosa produces several pigments all produced by a biosynthetic pathway: pyocyanin, 1-hydroxyphenazine, phenazine-1-carboxamide, 5-methylphenazine-1-carboxylic acid betaine, and aeruginosin A. Two operons are involved in phenazine biosynthesis: phzA1B1C1D1E1F1G1 and phzA2B2C2D2E2F2G2. These operons convert a chorismic acid to the phenazines mentioned above. Three key genes, phzH, phzM, and phzS convert phenazine-1-carboxylic acid to the phenazines mentioned above. Though phenazine biosynthesis is well studied, questions remain as to the final structure of the brown phenazine pyomelanin.[6],[7],[8]

When pyocyanin biosynthesis is inhibited, a decrease in *P. aeruginosa* pathogenicity is observed in vitro .This suggests that pyocyanin is most responsible for the initial colonization of *P. aeruginosa* in vivo.[9],[10]

Triggers:

With low phosphate levels, *P. aeruginosa* has been found to activate from benign symbiont to express lethal toxins inside the intestinal tract and severely damage or kill the host, which can be mitigated by providing excess phosphate instead of antibiotics.[10],[11]

Plants and invertebrates:

In higher plants, P. aeruginosa induces soft rot, for example in Arabidopsis thaliana (Thale cress) and Lactuca sativa (lettuce). It is also pathogenic to invertebrate animals, including the nematode Caenorhabditis elegans, the fruit fly Drosophila and the moth Galleria mellonella.The associations of virulence factors are the same for plant and animal infections.[10],[12],[13]

Diagnosis:

Depending on the nature of infection, an appropriate specimen is collected and sent to a bacteriology laboratory for identification. As with most bacteriological specimens, a Gram stain is performed, which may show Gram-negative rods and/or white blood cells. *P. aeruginosa* produces colonies with a characteristic "grape-like" or "fresh-tortilla" odor on bacteriological media. In mixed cultures, it can be isolated as clear colonies on MacConkey agar (as it does not ferment lactose) which will test positive for oxidase. Confirmatory tests include production of the blue-green pigment pyocyanin on cetrimide agar and growth at 42 °C.[1],[2],[3]

When *P. aeruginosa* is isolated from a normally sterile site (blood, bone, deep collections), it is generally considered dangerous, and almost always requires treatment.[citation needed] However*, P. aeruginosa* is frequently isolated from nonsterile sites (mouth swabs, sputum, etc.), and, under these circumstances, it may represent colonization and not infection. The isolation of P. aeruginosa from nonsterile specimens should, therefore, be interpreted cautiously, and the advice of the physician/pharmacist should be sought prior to starting treatment, often no treatment is needed.[5],[6],[9]

Production of pyocyanin, water-soluble green pigment of *P. aeruginosa* (left tube) 

Identification:

*P. aeruginosa* is a Gram-negative, aerobic (and at times facultatively anaerobic), bacillus with unipolar motility. It has been identified as an opportunistic pathogen of both humans and plants. *P. aeruginosa* is the type species of the genus Pseudomonas.[7]

In certain conditions, *P. aeruginosa* can secrete a variety of pigments, including pyocyanin (blue-green), pyoverdine (yellow-green and fluorescent), and pyorubin (red-brown). These can be used to identify the organism.[8],[9]

*P. aeruginosa* is often preliminarily identified by its pearlescent appearance and grape-like or tortilla-like odor in vitro. Definitive clinical identification of *P. aeruginosa* often includes identifying the production of both pyocyanin and fluorescein, as well as its ability to grow at 42 °C. *P. aeruginosa* is capable of growth in diesel and jet fuels, where it is known as a hydrocarbon-using microorganism, causing microbial corrosion.[51] It creates dark, gellish mats sometimes improperly called "algae" because of their appearance.[12]

Table(1): biochemical tests for *P. aeruginosa*

|  |  |
| --- | --- |
| Test | Result |
| Gram stain | \_ |
| Oxidase | + |
| Indole production | \_ |
| Methyl red | \_ |
| Voges \_ Proskaeur | \_ |
| Citrate | + |
| Hydrogen sulfide production | \_ |
| Urea hydrolysis | + |
| Phenylalanine ,Deaminase | \_ |
| Lysine Decarboxylase | \_ |
| Motility | + |
| Gelatin hydrolysis | + |
| Acid from lactose | \_ |
| Acid from glucose | + |
| Acid from maltose | \_ |
| Acid from mannitol | + |
| Acid from sucrose | \_ |
| Nitrate reduction | + |
| DNAse | \_ |
| Lipase | + |
| Pigment | + {bluish green pigmentation} |
| Catalase | + |

Materials and methods

Specimen collection: 60 swab specimens were collected from different sites ,( sputum , urine, otitis media, ,vaginal swab ,throat ,skin) . Information about their age, and antibiotic usage was taken into consideration. Each swab taken carefully from the site of infection and placed in tubes containing readymade media to maintain the swab wet during transferring to laboratory. [9],[10],[11]

Each specimen was inoculated on Pseudomonas isolation agar. All plates were incubated aerobically in incubator at 37ºC for 24 hrs. All cultures were grown on

nutrient or blood agar plates, All cultures were checked biochemically and stains for flagella.[14],[16]

were made on all doubtful strain of the microorganisms, 0.2 ml of the a-naphthol solution and 0.3 ml of p-aminodimethylaniline oxalate were added to each tube. The tubes were immediately shaken vigorously to insure mixing and the thorough oxygenation of the culture. The appearance of a blue color is indicative of the presence of cytochrome oxidase in the bacterial cells. To detect the presence of the enzyme in microorganisms cultured on agar plates, equal amounts of the above reagents are mixed and several drops allowed to flow over isolated colonies.[16],[17]

Broth cultures of *P. aeruginosa* gave a positive reaction (blue color) in 15 to 30 sec, indicating a readily

available high cellular concentration of cytochromeoxidase. All of the other stock cultures werenegative when tested for the presence of cytochromeoxidase by this method. As partial evidencethat this reaction is due to an oxidativemechanism present in *P. aeruginosa* the resultsshow that KCN inhibited the formation of indophenolblue by this microorganism. Colonies of P. aeruginosa on blood or nutrient agar turn blue in 15 to 30 sec when exposed to asolution containing equal parts ofa-naphthol andp-aminodimethylaniline oxalate, whereas the coloniesof other gram-negative bacilli are unaffected.[18],[19]

By this procedure it has been possible toidentify and isolate colonies of P. aeruginosa from a plate containing a mixed population.[19],[20]



  

Blood agar and show colonies of *p.aeruginosa*

Prevention

Probiotic prophylaxis may prevent colonization and delay onset of Pseudomonas infection in an ICU setting. Immunoprophylaxis against Pseudomonas is being investigated. The risk of contracting P. aeruginosa can be reduced by avoiding pools, hot tubs, and other bodies of standing water; regularly disinfecting and/or replacing equipment that regularly encounters moisture (such as contact lens equipment and solutions); and washing one's hands often (which is protective against many other pathogens as well). However, even the best hygiene practices cannot totally protect an individual against P. aeruginosa, given how common P. aeruginosa is in the environment. [20],[21],[22]

Experimental therapies:

Phage therapy against P. aeruginosa has been investigated as a possible effective treatment, which can be combined with antibiotics, has no contraindications and minimal adverse effects. Phages are produced as sterile liquid, suitable for intake, applications etc. Phage therapy against ear infections caused by P. aeruginosa was reported in the journal Clinical Otolaryngology in August 2009. [22],[23],[24]

*Treatment:*

Many *P. aeruginosa* isolates are resistant to a large range of antibiotics and may demonstrate additional resistance after unsuccessful treatment. It should usually be possible to guide treatment according to laboratory sensitivities, rather than choosing an antibiotic empirically. If antibiotics are started empirically, then every effort should be made to obtain cultures (before administering first dose of antibiotic), and the choice of antibiotic used should be reviewed when the culture results are available.[25],[26],[27]

Due to widespread resistance to many common first-line antibiotics, carbapenems, polymyxins, and more recently tigecycline were considered to be the drugs of choice; however, resistance to these drugs has also been reported. [28],[29]

Despite this, they are still being used in areas where resistance has not yet been reported. Use of β-lactamase inhibitors such as sulbactam has been advised in combination with antibiotics to enhance antimicrobial action even in the presence of a certain level of resistance. Combination therapy after rigorous antimicrobial susceptibility testing has been found to be the best course of action in the treatment of multidrug-resistant *P. aeruginosa*. Some next-generation antibiotics that are reported as being active against *P. aeruginosa* include doripenem, ceftobiprole, and ceftaroline.[29],[30]

 However, these require more clinical trials for standardization. Therefore, research for the discovery of new antibiotics and drugs against *P. aeruginosa* is very much needed. Antibiotics that may have activity against P. aeruginosa include: aminoglycosides (gentamicin, amikacin, tobramycin, but not kanamycin) quinolones (ciprofloxacin, levofloxacin, but not moxifloxacin) cephalosporins (ceftazidime, cefepime, cefoperazone, cefpirome, ceftobiprole, but not cefuroxime, cefotaxime, or ceftriaxone) antipseudomonal penicillins: carboxypenicillins (carbenicillin and ticarcillin), and ureidopenicillins (mezlocillin, azlocillin, and piperacillin). *P. aeruginosa* is intrinsically resistant to all other penicillins. carbapenems (meropenem, imipenem, doripenem, but not ertapenem)

polymyxins (polymyxin B and colistin) monobactams (aztreonam)

As fluoroquinolone is one of the few antibiotics widely effective against *P. aeruginosa*, in some hospitals, its use is severely restricted to avoid the development of resistant strains. On the rare occasions where infection is superficial and limited (for example, ear infections or nail infections), topical gentamicin or colistin may be used. [30],[31],[32]

*Antibiotic susceptibility test of P. aeruginosa:*

The agar diffusion test. isolates of *P. aeruginosa* were examined and number of different types of specimen were collected. Microbial sensitivity testing was done using disk diffusion test with Pseudomonas species .[32],[33]

It is a test of the antibiotic sensitivity of bacteria. It uses antibiotic discs to test the extent to which bacteria are affected by those antibiotics. In this test, wafers containing antibiotics are placed on an agar plate where bacteria have been placed, and the plate is left to incubate. If an antibiotic stops the bacteria from growing or kills the bacteria, there will be an area around the wafer where the bacteria have not grown enough to be visible. This is called a zone of inhibition.[33],[34]

The procedure:

1\_Using an aseptic technique, place a sterile swab into the broth remove of a specific organism and then gentlythe excess liquid by gently pressing or rotating the swab against the inside of the tube culture .[30],[31]

 2\_ Using the swab, streak the Mueller-Hinton agar plate to form a bacterial lawn :

a-To obtain uniform growth, streak the plate with the swab in one direction, rotate the plate 90°and streak the plate again in that direction.

b-Repeat this rotation 3 times.

3\_Allow the plate to dry for approximately 5 minutes.

4\_Use an antibiotic disc dispenser to dispense discs containing specific antibiotics onto the plate.

5-Using a flame-sterilized forceps, gently press each disc to the agar to ensure that the disc is attached to the agar.

6\_Plates should be incubated overnight at an incubation temperature of 37 °C .

7\_Measure the zone of inhibition and see the standard table to compare.

*The results:*

The samples(60) were planting on the media , the dishes were incubated at a temperature of 37 Celsius and within 24 hours, and has pursued growth until noon, and then has the purification and diagnosis process occur . Where the results of the study showed that 20 samples gave a positive result *for p. aeruginosa* , while 40 samples gave a negative result, the negative result due to the presence of fungal pathogens or viral or bacterial.The highest number of Pseudomonas infections was found in urine, followed by pus and sputum. Pseudomonas species demonstrated marked resistance against monotherapy of penicillins, cephalosporins, fluoroquinolones, tetracyclines and macrolides. Only combination drugs like Ticarcillin + Clavulanic acid, Piperacillin + Tazobactum, Cefoperazone + Sulbactum, Cefotaxime + Sulbactum, Ceftriaxome + Sulbactum and monotherapy of amikacin showed higher sensitivity to Pseudomonas infections; however, the maximum sensitivity was shown by the Carbapenems.[34],[35]

*RESISTANCE CHALLENGES FOR TREATMENT OF P. aeruginosa:*

*P. aeruginosa* presents a serious therapeutic challenge for treatment of both community-acquired and nosocomial infections, and selection of the appropriate antibiotic to initiate therapy is essential to optimizing the clinical outcome . Unfortunately, selection of the most appropriate antibiotic is complicated by the ability of *P. aeruginosa* to develop resistance to multiple classes of antibacterial agents, even during the course of treating an infection. Epidemiological outcome studies have shown that infections caused by drug-resistant *P. aeruginosa* are associated with significant increases in morbidity, mortality, need for surgical intervention, length of hospital stay and chronic care, and overall cost of treating the infection . [33],[34],[35],[36]

Even more problematic is the development of resistance during the course of therapy, a complication which has been shown to double the length of hospitalization and overall cost of patient care. *P. aeruginosa* can develop resistance to antibacterials either through the acquisition of resistance genes on mobile genetic elements (i.e., plasmids) or through mutational processes that alter the expression and/or function of chromosomally encoded mechanisms. Both strategies for developing drug resistance can severely limit the therapeutic options for treatment of serious infections.[36],[37]

*Antibacterial Resistance Trends*

*P. aeruginosa* exhibits the highest rates of resistance for the fluoroquinolones, with resistance to ciprofloxacin and levofloxacin ranging from 20 to 35%. [35]

. it is difficult to draw any strong conclusions about trends of resistance to various β-lactams. Among the aminoglycosides, most studies have focused on gentamicin, with resistance rates ranging from 12 to 22%. Gentamicin was the least active of the aminoglycosides, with lower rates of resistance being reported for tobramycin and amikacin in most studies. Not only are rates of resistance to individual drugs or drug classes a concern, but the prevalence of multidrug-resistant strains (resistant to three or more drug classes) is an even more serious therapeutic challenge .[36],[37]

The highest prevalence of multidrug-resistant strains was observed among isolates from lower respiratory tract infections, whereas the lowest prevalence was observed among isolates from upper respiratory tract infections. Not surprisingly, multidrug-resistant strains were isolated more frequently from ICU and nursing home patients.[37]

Results and Discussion:

Isolation and identification of *P. aeruginosa:*

 Out of the 60 samples, only 20(33.33%) isolates were belonged to *P. aeruginosa*, while other 40(66.66)% isolates represented other bacterial genera. The most isolates were obtained from ear swab 8(13.33)% , urine 6(10)%,sputum (3.33%) ,while throat(1.66)%, skin(1.66)%, and vaginal swab(1.66)%. The results are shown in table (1). The preliminary cultural diagnosis selective media is Pseudomonas isolation agar (PIA) for *P. aeruginosa* which appear circular mucoid smooth colonies with emits sweat grape odor , and then cultured on blood agar . Most isolates appear β-hemolysis on blood agar while others isolates were non hemolysis. All isolates grew on MacConkey agar, but did not ferment lactose sugar. All the isolate grew on the Muller- Hinton agar which produce the diagnostic pigment. The pigment varied from yellowish-green to bluish green and also the isolates produced a sweat grape-like odor. In this study the biochemical tests were carried out and the result compared with standard results.[34],[36]

 Table(2) :different cases of *p. aeruginosa* for culture

|  |  |  |  |
| --- | --- | --- | --- |
| Patient | Age | Specimen | result |
| P1 | 20 years | Ear swab | Growth of *p.aeruginosa* |
| P2 | 16 years | Urine  | Growth  |
| P3 | 25 years | Abscess | Growth  |
| P4 | 18 years | Ear swab | Growth  |
| P5 | 16 years | Urine | Growth  |
| P6 | 39 years  | Vaginal swab | Growth  |
| P7 | 60 years | Ear swab | Growth  |
| P8 | 8years | Ear swab | Growth  |
| P9 | 23 years | Skin | Growth  |
| P10 | 41 years | Urine | Growth  |
| P11 | 15 years | Urine | Growth  |
| P12 | 20 years | Ear swab | Growth  |
| P13 | 20 years | Sputum | Growth  |
| P14 | 60 years | Throat | Growth  |
| P15 | 12 years  | Urine | Growth  |
| P16 | 60 years | sputum | Growth  |
| P17 | 21 years | Ear swab | Growth  |
| P18 | 22 years | Ear swab | Growth  |
| P19 | 16 years  | Ear swab | Growth  |
| P20 | 30 years | Urine | Growth  |

 *Discussion sensitive test results*

*Obvious from the table that the pseudomonas aeruginosa show mixed response to antibiotics :-*

*Carbapenem (imipenem)*

S=75%, The binding affinities of imipenem to penicillin-binding proteins ( PBSs ) of Pseudomonas aeruginosa were determined by two different methods in which competition with [14C]benzylpenicillin for the binding sites was measured.[33],[34]

 By both methods imipenem was shown to have very high binding affinities to PBPs-2 and -4 in P. aeruginosa, and appreciable affinities to most of their other major PBPs. But higher concentrations of imipenem were required for binding to the PBPs-3 in these bacteria*.* *The antibiotic also seemed to cause strong inhibition of the transglycosylase* activity of PBP-1A by some unknown mechanism.[34],[35]

 It inhibited the transpeptidase activity of PBP-3 only weakly, which is consistent with the findings that it had low binding affinity to PBP-3 and did not inhibit septum formation by the cells.[36],[37]

R=0%

*Fluoroquinolones(ciprofloxacin, ofloxacin ,norfloxacin, levofloxacin, temafloxacin)*

S=5%, 35%,60%, 30%, 15% .respectively: The mode of action of quinolones involves interactions with both DNA gyrase, the originally recognised drug target, and topoisomerase IV, a related type II topoisomerase. The formation of the ternary complex of quinolone, DNA, and either DNA gyrase or topoisomerase IV occurs through interactions in which quinolone binding appears to induce changes in both DNA and the topoisomerase that occur separately from the DNA cleavage that is the hallmark of quinolone action.[35],[36],[37]

R=0, 5%,0%,0%,15% respectively : Resistance to fluoroquinolones typically arises as a result of alterations in the target enzymes (DNA gyrase and topoisomerase IV) and of changes in drug entry and efflux. augment resistance further, so that the most-resistant isolates have mutations in several genes. Resistance to quinolones can also be mediated by plasmids that produce the Qnr protein, which protects the quinolone targets from inhibition.[35],[36]

*Penicillines (pipracillin, flucloxacillin ,nafcillin)*

S=20%, 10%,0%respectively: Penicillin kills susceptible bacteria by specifically inhibiting the transpeptidase that catalyzes the final step in cell wall biosynthesis, the cross-linking of peptidoglycan.[37]

R=15%,25%,15% respectively: Bacteria generate antibiotic resistance through a number of mechanisms. Some bacteria can become resistant to penicillin by producing beta-lactamase, a bacterial enzyme that destroys the beta-lactam ring of penicillin and makes it ineffective.[36],[37]

*Cephalosporines(ceftriaxone ,cefixime ,cefepime, cefpodoxime)*

S=60%.,10%,10%,10% respectively: Cephalosporins are bactericidal and have the same mode of action as other β-lactam antibiotics (such as penicillins), but are less susceptible to β-lactamases. Cephalosporins disrupt the synthesis of the peptidoglycan layer forming the bacterial cell wall. The peptidoglycan layer is important for cell wall structural integrity. The final transpeptidation step in the synthesis of the peptidoglycan is facilitated by penicillin-binding proteins (PBPs) Beta-lactam antibiotics mimic the D-Ala-D-Ala site, thereby irreversibly inhibiting PBP crosslinking of peptidoglycan.[34],[35],[36]

R=10%,30%,5%,5% respectively: Resistance to cephalosporin antibiotics can involve either reduced affinity of existing PBP components or the acquisition of a supplementary β-lactam-insensitive PBP.[36],[37]

*Macrolids (clarithromycin ,erythromycin ,azithromycin )*

S=20%, 5%,10% respectively: de of Action

Macrolides exert their antibiotic effect by binding irreversibly to the 50S subunit of bacterial ribosomes. Ribosomes are the protein factories of the cell, and by binding to the ribosome, macrolides inhibit translocation of tRNA during translation (the production of proteins under the direction of DNA). [35],[36]

R=25%,20%,0% respectively: The primary means of bacterial resistance to macrolides occurs by post-transcriptional methylation of the 23S bacterial ribosomal RNA. resistance include ribosomal modification, efflux of the antibiotic, and drug inactivation, results in a variety of phenotypes of resistance.[34],[35]

*Glycopeptides (vancomycin)*

S=5%:The bactericidal action of vancomycin results primarily from inhibition of cell-wall biosynthesis. Specifically, vancomycin prevents incorporation of N-acetylmuramic acid (NAM)- and N-acetylglucosamine (NAG)-peptide subunits from being incorporated into the peptidoglycan matrix; which forms the major structural component of cell walls. [36],[37]

R=45%;Resistance has manifested itself largely through the expression of genes that encode proteins that reprogram cell wall biosynthesisThe glycopeptides bind to the peptidyl-D-Ala4-D-Ala5 extremity of peptidoglycan precursors and block by steric hindrance the essential glycosyltransferase and D,D-transpeptidase activities of the penicillin-binding proteins (PBPs).[34],[35],[36]

*Aminoglycoside(gentamicin)*

S=25%: The mechanism of action of aminoglycosides depends on the inhibition of polypeptide synthesis. In fact, some of them such as Gentamicin are believed to cause codon misreading, by increasing the incorporation of certain aminoacids into polypeptide in the ribosome-polyribonucleotide system. Their primary site of action seems to be located in the bacterial ribosome system. When polypeptide synthesis is directed by endogenous messenger in cell-fre extracts, the antibiotic stimulates the incorporation of some amino acids into protein.[36],[37]

R=0:; There are three mechanisms of aminoglycoside resistance: reduced uptake or decreased cell permeability, alterations at the ribosomal binding sites, or production of aminoglycoside modifying enzymes.[35],[36],[37]

Reduced uptake or decreased cell permeability:

Some strains of Pseudomonas aeruginosa and other gram-negative bacilli exhibit aminoglycoside resistance due to a transport defect or membrane impermeabilization.  This mechanism is likely chromosomally mediated and results in cross-reactivity to all aminoglycosides.

Altered Ribosome Binding Sites:

Enzymatic Modification:

      Enzymatic modification is the most common type of aminoglycoside resistance. Over 50 different enzymes have been identified.  Enzymatic modification results in high-level resistance.  The genes encoding for aminoglycoside modifying enzymes are usually found on plasmids and transposons.  Most enzyme-mediated resistance in gram-negative bacilli is due to multiple genes.  It is hypothesized that the enzymes are derived from organisms that make the aminoglycoside or from the mutation of genes that encode the enzymes involved in cellular respiration.[35],[36]

 Table(3) :Sensitive test of *p. aeruginosa* isolates

|  |  |  |
| --- | --- | --- |
| Antibiotic | No. isolates of sensitive (%) | No. isolates of resistance (%) |
| IMI | 75% | 0 |
| PRL | 20% | 15% |
| NOR | 60% | 0 |
| CTX | 60% | 10% |
| OFX | 35% | 5% |
| C | 20% | 25% |
| VA | 5% | 45% |
| CFM | 10% | 30% |
| FOX | 10% | 25% |
| LEV | 30% | 0 |
| TM | 15% | 15% |
| CPM | 10% | 5% |
| CPD | 10% | 5% |
| AZM | 10% | 0 |
| CIP | 5% | 0 |
| NA | 0% | 15% |
| E | 5% | 20% |
| GM | 25% | 0 |

IMI=imipenem PRL*=*pipracillin NOR=norfloxacin CTX=ceftriaxone OFX=ofloxacin C=clarithromycin VA=vancomycin CFM=cefixime FOX=flucioxacillin LEV=levofloxacin TM=temafloxacin CPM=cefepime CPD=cefpodoxime AZM=azithromycin CIP=ciprofloxacin NA=nafcilin E=erythromycin GM=gentamycin

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