Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/6076
DOI URL: http://dx.doi.org/10.21474/IJAR01/6076

RESEARCH ARTICLE

ANTIBIOTIC ACTION OF PYOCYANIN ON SOME PATHOGENIC BACTERIA ISOLATED FROM WOUND INFECTION.

Sabiha Sharif salih and Lana M A. Mohammed.
Sulaimani Polytechnic University, Technical college of Health, Medical Laboratory Department.

Manuscript Info

Manuscript History

Received: 16 October 2017
Final Accepted: 18 November 2017
Published: December 2017

Key words:-
pyocyanin, *Pseudomonas aeruginosa, Klebsella oxytoca, Proteus mirabilis, Escherichia coli, staphylococcus aureus*.

Abstract

Bacteria *Pseudomonas aeruginosa* has ability to survive in various niches due to its capacity of production and releasing some compounds, which act as inhibitory effect of bacteria, fungi and protozoa, etc. It has been reported that the synthesis of pyocyanin by many strains could be the reason for *P. aeruginosa* to survive and compete other microorganisms in the nature. Antibacterial activity of pigment pyocyanin that extracted from *P. aerogenosa* has been studied using gram negative bacteria *Klebsiella oxytoca, Proteus mirabilis, Escherichia coli* and gram positive bacteria *staphylococcus aureus.* At the same time, antibiotic susceptibility test was performed for all bacteria using 12 different antibiotics in the absence and presence of pyocyanin pigment. As a result, all bacteria were sensitive in the presence of pyocyanin, while all bacteria were resistant to used antibiotics without pyocyanin except *Proteus mirabilis*, which was sensitive to both antibiotics Tetracycline and Amikacin. However, the results of bacterial sensitivity test to antibiotics in the presence of pyocyanin were variable.

Introduction:-

Pseudomonas aeruginosa is wide spread opportunistic bacterium in the nature. It can be found in aquatic and terrestrial environments (Paerl, 1989; Marshall *et al*., 1986). *P. aeruginosa* considers as pathogenic bacteria in human with impaired immune system (Nester and Anderson, 2001). *P. aeruginosa* has ability to survive in various niches due to its capacity of production and releasing some compounds, which act as inhibitory effect of bacteria, fungi and protozoa etc. It has been reported that the synthesis of pyocyanin by many strains could be the reason for *P.aeruginosa* to survive and compete other microorganisms in the nature (Hoadley *et al*., 1982; McGowan *et al*., 1988). Pyocyanin is extracellular blue-green phenazine pigment that produced by *Pseudomonas aeruginosa* strains as a secondary metabolite (Schaffer and Melanie, 2015). Pyocyanin considers as *P.aeruginosa's* virulence factor that play role in human lung infection. Pyocyanin also can be useful in different industrial applications such as; food mining, pharmaceuticals, textiles, leather and other industries (Schaffer and Melanie, 2015). According to several researches pyocyanin can be act as an active antibiotic against different antibiotic resistant pathogenic bacteria. Staphylococci are non-motile, non-spore forming facultative anaerobic bacteria. *Staphylococcus aureus* is Gram-positive bacteria that known as a most pathogenic species of Staphylococcus genus. *S. aureus* can cause infection in both healthy human and human with impaired immune system. \It occurs naturally on human skin and nasopharynx and it can cause different local infections on skin, in nose, urethra, vagina and gastrointestinal tract (Harris *et al*., 2001). Recently *S. aureus* has been one of the most studied species of staphylococcus according to continuously

Corresponding Author:- Lana MA. Mohammed.
Address:- Sulaimani Polytechnic Technical college of Health, Medical Laboratory.

increasing its antibiotic resistance (Costa *et al*., 2013). *Proteus mirabilis* is Gram-negative bacteria that clinically known as bacteria that can swarm on agar surface and produce urease enzyme (Schaffer and Melanie, 2015). *P. mirabilis* consider one of the pathogenic bacteria that able to cause various human infections such as; blood stream, intra-abdominal and urinary tract infections, which are most known *proteus* infection. It has been known that *P. mirabilis* were resistance to wide spectrum range of antibiotics including cephalosporins (Sohn *et al*., 2011).

Escherichia coli is Gram-negative bacteria that considers as normal flora living in human and animal digestive system. However, *E. coli* can be pathogenic and causes diseases which due to virulence factor acquisition (Zinnah *et al*., 20017). Pathogenic strains of *E. coli* cause diseases because of their ability for production of shiga toxin. Contaminated meat products and ground beef consumption are considering as the main source of *E. coli* infection in human (Law, 2000).

Klebsiella spp. is Gram-negative opportunistic bacteria that can cause different human and animal infections such as; wound infections, sepsis in newborns, urinary tract infections and bacteremia (Stock and Wiedemann, 2001). *Klebsiella oxytoca* are frequently isolated from nosocomial infections. *K. oxytoca* can be the source of various animal infections such as; urinary and respiratory tract infection, mastitis and sepsis.

The main aim of this study is extraction of pyocyanin pigment from bacteria *P. aeruginosa* and to determine the antibacterial activity of pyocyanin against some human pathogenic bacteria that isolated from wound infection. Simultaneously, 12 different commercial antibiotics were tested for diagnosing sensitivity of the used pathogenic bacteria to these antibiotics in the presence and absence of pyocyanin.

Materials and Methods:-

1.1. Antibiotic susceptibility determination:

The antibiotics were purchased from sigma german company and they included; Ampicillin, Cefotaxime, Erythromycin, Gentamycin, Penicillin, Trimethoprim, Tetracycline, Streptomycin, Amikacin, Ciprofloxacin, Nalidixic acid and Rifampicin. The antimicrobial resistance phenotypes of all isolated bacteria were determined using agar plate dilution method. The final concentration of the antibiotics were determined and then added to the agar media and finally the mixtures were poured into petri-dishes. Streaking method was used to bacterial inoculation, after bacterial inoculation the petri-dishes were kept in the 37C incubator for 24hrs before recording the results (Atlas *et al*., 1995).

1.2. Isolation of bacteria:

Bacteria *P. aeruginosa, K. oxytoca, P. mirabili, E. coli* and *S. aureus* were isolated from wound infections in Sulaimaniyah teaching hospital. All bacteria samples were identified by Vitek analysis in Teaching hospital.

1.3. Minimum inhibitory concentration (MIC) determination of pyocianin:

Antibacterial activity of pyocyanin was tested using serial dilution of isolated bacterial inoculum. Bacterial samples were standardized by using previously prepared standard curve as recommended by (Cruick *et al*., 1975). Bacterial suspension were further diluted for obtaining $1x10^5$ CFU inoculum using spectrophotometer and counting viable cell on nutrient agar (Atlas *et al*., 1995).

1.4. Extraction of pyocyanin:

Nutrient broth media was inoculated with *P. aeruginosa* in order to isolation of pyocyanin pigment in the laboratory. After inoculation the broth culture were incubated in 37C for 24 hours with shaking and prior color changing the culture media was centrifuged at 10.000 rpm for 5 minutes. Chloroform (1:2) was used for pigment extraction and the aqueous phase was discarded. 0.2 N of HCl was used for solvent phase and followed by color changing. The pigment was further detected and characterized by using UV-visible spectrophotometer (UV T-1800). Maximum absorption was observed by sampling 2ml aliquot of the pigment (Saha *et al*., 2008).

Results:-

Antibacterial activity of pigment pyocyanin that extracted from *P. aerugenosa* has been studied using gram negative bacteria *K. oxytoca, P. mirabilis, E. coli* and gram positive bacteria *S. aureus*. At the beginning pyocyanin susceptibility test was performed for all used bacteria and the result showed that all bacteria were sensitive to the pyocyanin (Table 1). Later, antibiotic susceptibility test was done for gram positive and gram negative bacteria

using 12 different antibiotics, which were; Ampicillin , Cefotaxime, Erythromycin, Gentamycin, Penicillin, Trimethoprim, Tetracycline, Streptomycin, Amikacin, Ciprofloxacin, Nalidixic acid, Rifampicin. All bacteria were resistant to used antibiotic except *P. mirabilis*, which was sensitive to both Tetracycline and Amikacin (Table 2). After obtaining the previous results, antibacterial activity of pyocyanin was diagnosed in the present of the final concentration of 12 antibiotics. The results demonstrated that gram positive bacteria *S. aureus* were obviously sensitive for all used antibiotics in the presence of pyocyanin. Gram negative bacteria *E. coli* were sensitive for all antibiotics with pyocyanin except antibiotics Erythromycin and penicillin, which were resistant. Bacteria *P. mirabilis* were sensitive for all antibiotics with pyocyanin except antibiotics Erythromycin and Nalidic acid. However bacteria *K. oxytoca* showed variable responses to the antibiotics in the presence of pyocyanin. The bacteria were sensitive to Gentamycin, Rifampicin, Streptomycin, Ciprofloxacin, Cefotaxime, Trimethoprim, while they were resistant to Amikacin, Penicillin, Ampicillin, Tetracycline, Erythromycin and Nalidixic acid (Table 3).

Bacteria	**Sub MIC**	**Pyocianin**
Klebsiella oxytoca	**2000 µg/ml**	**S**
Proteus mirabilis	**2500 µg/ml**	**S**
Escherichia coli	**1000 µg/ml**	**S**
Staphylococcus aureus	**1500 µg/ml**	**S**

Table1:- sub-minimum inhibitory concentration value (µg/ml) of Pyocyanin against each bacterium *Klebsiella oxytoca,* **Proteus mirabilis,** *Escherichia coli and Staphyllococcus aureus*.

	Antibiotics											
Bacteria	**Amp.**	**Pn.**	**Gm.**	**Str.**	**Rif.**	**Cip.**	**Cef.**	**Nal.**	**Er.**	**Tri.**	**Te.**	**Amk.**
Klebsella oxytoca	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Proteus mirabilis	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**S**
Escherichia coli	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Staphyllococcusa ureus	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**

Table 2:- Sensitivity of bacteria bacterium Klebsiella oxytoca, *Proteus mirabilis,* Escherichia coli and Staphyllococcus aureus to the antibiotics; Ampicillin, Penicillin, Gentamycin, Streptomycin, Rifampicin, Ciprofloxacin, Cefotaxime, Nalidixic acid, Erythromycin, Trimethoprim, Tetracycline, Amikacin.

	Antibiotics											
Bacteria	**Amp.**	**Pn.**	**Gm.**	**Str.**	**Rif.**	**Cip.**	**Cef.**	**Nal.**	**Er.**	**Tri.**	**Te.**	**Amk.**
Klebsella oxytoca	**R**	**R**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**S**	**R**	**R**
Proteus mirabilis	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**S**	**S**	**S**
Escherichia coli	**S**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**S**	**S**	**S**
Staphylococcus aureus	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**

Table 3:- Sensitivity of bacteria *Klebsiella oxytoca, Proteus mirabilis, Escherichia coli* and *Staphylococcus aureus* to the 12 antibiotics in the presence of Pyocianin.

Discussion:-

According to the most published researches, our experimental results are in good agreement with following studies that showed antibacterial effect of pyocyanin on different pathogenic bacteria growth. The bacterial growth inhibitory effect of pyocyanin that isolated from *P. aeruginosa* has recognized earlier when respiratory activity of both bacteria *Vibrio cholerae* and *Staphylococcus aureus* were almost blocked in the presence of 500 ug/ml of pyocyanin (Schoental, 1952). It has been reported that *P. aeruginosa* has ability to produce various types of pigments that could act as an antibacterial against many pathogenic bacteria and pyocyanin consider one of them (Groscop and Brent, 1990). Antibacterial activity of pyocianin that extracted from 50 different *Pseudomonas* strains was tested against growth of *E. coli* and compared with antibiotic activity. The results showed that pyocyanin has strong inhibitory effect on *E. coli* though the bacterial inhibition zone increased from 2.7 mm to 13.3 mm in the presence of 20µl and 60µl of pyocyanin respectively (Aonofriesei and Crâsmaru, 2002). Another study investigated the antimicrobial effect of pyocyanin that produced by *P. aeruginosa* strains that isolated from clinical samples by using agar well diffusion method. The results showed that pyocyanin has bacterial inhibitory effect on *Bacillus sp.* with 31 mm, *E. coli* with 12 mm, Candida sp. with 12 mm (diameter of inhibition zone) with no antifungal effect (Özyürek Sezen *et al*., 2016). Different studies have been shown that pyocyanin has greater growth inhibitory effect

on gram positive bacteria than Gram negative bacteria. Gram negative bacteria Salmonella typhi, *P. mirabilis*, *E. coli*, *Acinetobacter sp*. were sensitive to pyocyanin, while *Klebsiella pneumonia*, *P. aeruginosa*, *P. vulgaris* were resistant to growth inhibitory effect of pyocyanin (El-Shouny, 2011; Sweedan, 2010). Other studied reported that pyocyanin has great inhibitory effect on growth of Gram positive bacteria such as *S. aureus*, *Bacillus subtilis* and Gram negative bacteria such as *E. coli, Klebsiella sp., S. typhi, Shigella sp., P. vulgaris* (El-Fouly, 2015; Norman, 2002). In addition to this, it has been recorded that Gram positive bacilli and cocci are more sensitive to antibacterial effect of pyocyanin than Gram negative bacilli, while all strains *P. aeruginosa* were highly resistant to pyocyanin (Baront and Rowe, 1981).

It has been concluded that pyocyanin produced by *P. aeruginosa* can be considered as a good antibacterial medicine to prevent the growth of used pathogenic bacteria. Moreover, bacterial sensitivity to different types of commercial antibiotics could be changed in the presence of pyocyanin pigment. It has been suggested that pyocyanin may have ability to cause changes in microbial electron transport respiratory chain and produces toxic oxygen free radical that cause microbial growth inhibition and death.

References:-

1. Aonofriesei F and Crâsmaru M. (2002). Antibacterial activity of pyocianin produced by some Pseudomonas strains isolated from seawater. Ovidius University, Faculty of Natural Sciences and Agriculture Sciences, Blvd. Mamaia 124.
2. Atlas r.m., brown a.e. and parks l.c. (1995). Laboratory manual experimental microbiology. mosby-yearbook inc., st. Louis.
3. Baront Stephen S. and Rowe John J., (1981). Antibiotic Action of Pyocyanin. Antimicrobial agents and chemotherapy. p. 814-820 Vol. 20, No. 6.
4. Costa Rita Ana, Batistão W. F. Deivid, Ribas M. Rosineide, Sousa Margarida Ana, Pereira M. Olivia and Botelho M. Claudia. (2013). Staphylococcus aureus virulence factors and disease. Microbial pathogens and strategies for combating them: science, technology and education (A. Méndez-Vilas, Ed.).
5. Cruick Shank, R;D Duguid; B.Pmarmition and R.H.A. Swain. (1975). Medical microbiology, the practice of medical microbiology. 12thed.,vol.1. Churchill living stone, london and new York.
6. El-Fouly M.Z., Sharaf A. M., Shahin A.A.M., El-Bialy H.A., (2015). Biosynthesis of pyocyanin pigment by Pseudomonas aeruginosa, J. Radiat. Res. Appl. Sci.,36-48. 8.
7. El-Shouny W.A., Al-Baidani A.R.H., Hamza W.T., (2011). Antimicrobial activity of pyocyanin produced by Pseudomonas aeruginosa isolated from surgical wound infections, Int. J. Pharm. Med. Sci.,1-7. 1.
8. Groscop, J.A., Brent, M.M. (1990). The effects of selected straines of pigmented microorganisms on small free-living ameobae. Can. J. Microbiol. 10: 579-584.
9. Harris. L.G., Foster.S.J., and Richards R.G. (2002).An Introduction To Staphylococcus aureus, and Quantifying S. aureus Adhesins In Relation To Adhesion To Biomaterials;Review. European cells and Materials Vol. 4. 39-60.
10. Hoadley, A.W., Ajello, G. (1982). Some characteristic of fluorescent pseudomonads isolated from surface waters and capable of growth at 41oC. Can. J. Microbiol. 18: 1769-1773.
11. Law, D. (2000).The History and Evolution of Escherichia coli O157 and other Shiga toxinproducing E. coli. 16:701-709.
12. Marshall, K.C., Stout, R., Mitchell, R. (1986). Mechanism of the initial events in the sorption of marine bacteria to surfaces. J. Gen. Microbiol.68: 337-348.
13. McGowan, R.P., Cheng, K.-L., Bailey, C.B.M., Costerton, J.W. (1988). Adhesion of bacteria to epithelial cell surfaces within reticulo-rumen of cattle. Appl. Environ. Microbiol. 35; 149-155.
14. Nester EW; Anderson DG. (2001). A human perspective In Microbiology, 3rd ed, McGraw-Hill companies, New Jersey, USA.
15. Norman R.S., Moeller P., McDonald T.J., Morris P.J., (2002). Effect of pyocyanin on a crude-oil degrading microbial community, Appl. Anviron. Microbiol., 4004-4011. 70.
16. Özyürek Sezen Bilen, Gür Sinem Diken and Bilkay Işıl Seyis. (2016). Investigation of Antimicrobial Activity of Pyocyanin Produced by Pseudomonas aeruginosa Strains Isolated from Different Clinical Specimens. S.B. Özyürek et al. / Hacettepe J. Biol. & Chem.,44 (1),1–6.
17. Paerl, W. (1989). Microbial attachment to particles in marine amd freshwater ecosystems. Microb. Ecol.2: 73-83.
18. Saha S., Thavas R., Jayalakshmi S., (2008). Phenazine pigments from Pseudomonas aeruginosa and their application as antibacterial agent and food colourants, Res. J. Microbiol.,122-128. 3.

19. Schaffer N. Jessica and Melanie M. Pearson. (2015). Proteus mirabilis and Urinary Tract Infections. Microbiol Spectr. 3(5)10.1128.
20. Schoental, B. (1952). The nature of the antibacterial agents present in Pseudomonas pyocyanea cultures. Brit. J. Exp. Pathol. 22: 137-147.
21. Sohn Kyung Mok, Kang Cheol-In, Joo Eun-Jeong, Ha Young Eun, Doo Ryeon, Chung Kyong Ran Peck, Lee Nam Yong and Song Jae-Hoon. (2011). Epidemiology of Ciprofloxacin Resistance and Its Relationship to Extended-Spectrum β-Lactamase Production in Proteus mirabilis Bacteremia. Original Article. 26.1.89.
22. Stock Ingo and Wiedemann. (2001). Natural antibiotic susceptibility of Klebsiella pneumoniae, k. oxytca, K. planticola, K. orinthinolytica and K. terrigena strains. J. Med. Microbiol. Vol. 50, 396-406.
23. Sweedan E.G., (2010). Study the effect of antibiotics on pyocyanin production from Pseudomonas aeruginosa and pyocyanin as antibiotic against different pathogenic bacteria, J. University of anbar for pure science, 4.
24. Zinnah M. A., Bari M. R., Islam M. T., Hossain M. T., Rahman M. T., Haque M. H., Babu S. A. M., Ruma R. P. and Islam M. A. (2007). Characterization Of Escherichia coli Isolated From Samples Of Different Biological And Environmental Sources. Bangl. J. Vet. Med. 5 (1 & 2): 25–32.