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RESEARCH ARTICLE

USING DIFFERENT CURING METHODS TO DETERMINE RESISTANCE OF ANTIBIOTICS IN *Streptococcus pyogenes*

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ABSTRACT

Fifteen isolates of *Streptococcus pyogenes was* isolated and activated from tonsillitis in Muqdadiya city/Iraq. The sensitively of these isolates have been tested against (15) antibiotics. The results showed that highest resistance among Amikacin, Ampicillin and Trimethoprim with percentage 100%, while the lowest resistance was recorded for Penicillin, Imipenem, Chloramphenicol and Vancomycin with 0%. Results of investigation of some virulence factors of *S.pyogenes* indicate that all isolates produce hemolysin enzyme with 100% and that all isolates surrounded by capsule with percentage 100% and uncapable to producing bacteriocins, while (14) isolates (93.3%) are able to Dnase producer, whereas (13) isolates (86.6%) were Bio film producer, in addition to (11) isolates (73.3%) had the ability to produce cysteine protease enzyme, on other hand (9) isolates (60%) were able to streptokinase production. The results of plasmid content indicate that all isolates contain single large plasmid band. Curing of plasmids was conducted by use of three materials included acridine orange, ethidium bromide and sodium dodecyl sulfate, the acrdine orange showed strongest curing material and the plasmid lost at concentration (256 µg/ml) and plasmid lost at by ethidium bromide with concentration (512 µg/ml), whilst sodium dodecyl sulfate was the weaker material and the plasmid lost at concentration (2000 µg/ml).

INTRODUCTION

Group A Streptococcus (GAS; *Streptococcus pyogenes)* is a Gram-positive human pathogen which typically colonizes the throat or skin of the host. While normally causing only superficial cases of Pharyngitis or impetigo, GAS is readily capable of infecting otherwise sterile deep tissue. GAS diseases are underpinned by an extensive repertoire of virulence determinants that are differentially regulated in direct response to a battery of environmental signals within the host. The trigger for rapidly progressive invasive GAS disease has also been linked to the capacity of GAS to hijack host molecules for redeployment as virulence factors (Carapetis *et al*., 2005). *Streptococci* have virulence factors especially *Streptococcus pyogenes* and this virulence factors play important role in the pathogenesis of bacteria including capsule, M protein that help bacteria in the resistance of Phagocytosis process (Cunnighaham, 2000). *S.pyogenes* have the ability to produce Extra cellular enzymes such as streptokinase and hyalodorinase with the extensive use of third and fourth generation cephalosporins as an important component of empirical therapy in intensive care units and high risk world (Angelescu and Apostol, 2001), resistance to these drugs has become a major problem all over the world (kalin, 1999). Resistance has developed in bacteria by possessing extended spectrum beta – lactamase (ESβLs) capable of hydrolyzing these newer cephalosporins (Pagani *et al*., 2002). The aim of this study to determine the sensitive of antibiotics, virulence factors and curing of plasmids by three materials such as Acridine orange, ethidium bromide, sodium dodecyl sulfate

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MATERIALS AND METHODS

Activation of *Streptococcus pyogenes*

Fifteen *Streptococcus pyogenes* isolated and activated from tonsillitis from different hospital in Muqdadiya city during a period from September 2012 to December 2012 and these isolates have been activated by brain heart infusion medium at 37 C^0, 24 hour at 120 r.p.m.

Antimicrobial susceptibility test

Fifteen antibiotics including Beta lactam group, Quinolones group and aminoglycoside group have been tested in order to test the sensitivity of *Streptococcus pyogenes* by using the Muller Hinton agar plates and add blood to this plates. Results

of susceptibility test have been recorded according to the guidelines of the National Committee for Clinical Laboratory standers (NCCLS, 2002).

Determination of virulence factors

Some virulence factors have been studied including Hemolysin, capsule, streptokinase, DNase, cysteine protease, biofilim, Bacteriocin and Streptolysin O by using different media

Plasmid profile (Plasmid DNA analysis)

Plasmid DNA of the one isolate have been extracted by using the Pure Yield™ Plasmid Miniprep Kit (Promega U.S.A). Plasmid DNA were analyzed by electrophoresis on 0.7% agarose gel containing 0.5µg of ethidium bromide per ml (Sambrook *et al*., 1989) and pass the electricity (7 volt⁄cm2) for (1-1.5) hour until the pigment arrive to other side of the gel agarose. The agarose have been tested by using ultra violate transilluminator in wave length (336nm).

Curing of plasmid DNA

Curing have been conducted by using different concentrations of Acridin orange and ethidium bromide and sodium dodecyl sulfate (16, 32, 64, 128, 256, 512, 1024, 2000, 2500, 3000) µg/ml (Sambrook *et al*., 1989; Trevorse, 1986).

RESULTS AND DISCUSSION

Determination antimicrobial susceptibility test of *Streptococcus pyogenes*

The sensitivity of these isolates have been tested against (Riley *et al*., 2003) antibiotics. The results demonstrated that high resistance of Amikacin, Ampicillin, Trimethoprim with 100% Figure (1).

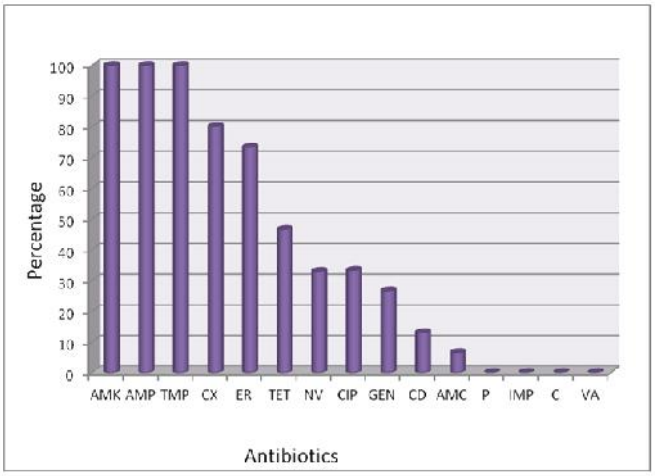

Fig. 1. Percentage of resistance *S.pyogenes* to antibiotics

This result has the agreement with previous study was done by (Al-Baghdady *et al*., 2006) who pointed out that resistance rates in *S.pyogenes* as 96.92%, resistance of bacteria to Amikacin may be due to the changes of the outer membrane permeability or have efflux system in both gram positive and gram negative bacteria. The resistance of Cloxacillin was 80%, while *S.pyogenes* resists erythromycin, tetracycline, novobiocin, with 73%, 46.6%, 33.3% respectively. The results showed that *S.pyogenes* resists ciprofloxacin with 33.3%, while the isolates resists Gentamycin, Clindamycin, Augmentin 26.6%, 13.3%, 6.6 respectively and the isolates sensitive to Penicillin, Imipenem, Chloramphenicol, Vancomycin with 100%. This resistance of different antibiotic attributed to the presence of multiple drug-resistant strains (Kayser *et al*., 2005). Antibiotic resistance probably developed by the transfer plasmid from drug resistant bacteria to sensitive bacteria or because of its propensity to develop resistance during therapy (Goering *et al*., 2008)

Determination of virulence factors of *Streptococcus pyogenes*

Biofilm production

Results showed that 86.6% of *Streptococcus pyogenes* have the ability to produce Biofilm this results have the agreement with (Doem *et al*., 2008) who indicated that 93% of isolates produce Biofilm and also agrees with (Baldassarri *et al*., 2006) who pointed out that 90% of isolates were producer to Biofilm. The positive results of this factor that the isolates appear as black colour have dry density Crystalline table (1) but the negative result that the colony appear with pink colour .

Bacteriocin production

The study illustrated that the isolates unable of producing bacteriocins table (1), this study agrees with (Mojani *et al*., 2006) who showed that isolates unable to produce bacteriocins. In this study used trypticase soy agar medium and added yeast extract by1% to increase Productictivity of Bacteriocins (Riley *et al*., 2003).

Capsule formation

The study showed that all isolates surrounded by capsule with percentage 100% table (1) and this results has the agreement with (Macfaddin, 2000), who demonstrated that fierce strains of bacteria contain capsule. The capsule play important role in pathogenicity of bacteria by resistance host defense because *S.pyogenes* contain hyalornate that Similar to hyalornate material of the connective tissue in the host and play role in the Hide bacterial antigens an Prevent the immune system know them (Wessels, 2006).

Hemolysin production

Results of investigation of some virulence factors of *S.pyogenes* referred to that all isolates are produce Hemolysin enzyme with percentage 100% Table (1). (Zunino *et al*., 1999) suggested that the Hemolysin not essential during early infection but this factor is important at late stage of infection.

Cysteine protease

This study showed that (73.3%) from isolates have the ability to produce Cysteine protease enzyme Table (2). This result agrees with (Whealer *et al*., 1991) who showed that 73% from isolates produce Cysteine protease.

Table 1. Virulence factors

No.of isolates	capsule	bacteriocin	biofilm	hemolysin
1	+	-	+	+
2	+	-	+	+
3	+	-	+	+
4	+	-	-	+
5	+	-	+	+
6	+	-	+	+
7	+	-	+	+
8	+	-	+	+
9	+	-	-	+
10	+	-	+	+
11	+	-	+	+
12	+	-	+	+
13	+	-	+	+
14	+	-	+	+
15	+	-	+	+

DNase production

This study showed that (93.3%) DNase producer Table (2) this study agrees with (20) who mentioned that 100% of isolates produce DNase. DNase has the ability to degrade host DNA lead to increase pathogenicity of *S.pyogenes*

Streptokinase

Results of investigation of streptokinase production illustrated that 60% of isolates produce this enzyme and this results has the agreement with (Razak *et al*., 2012), who mentioned that 66.6% from the isolates produce streptokinase and this result does not compatible with (Bhardwaj *et al*., 2013) who showed that 32.3% from isolates produce this enzyme Table (2). Streptokinase is a novel fibrinolytic protein produced by several species of streptococci. As a therapeutic, streptokinase can be used in the treatment of thromboembolic disorders where it dissolves a blood clot by the activation of plasminogen to plasmin. Specimens from infected throat can be an excellent source for the isolation of haemolytic organisms (Bhardwaj *et al*., 2013)

Table 2. Virulence factors including enzymes

Number of isolates	Cysteine protease	dnase	streptokinase
1	+	+	+
2	+	-	-
3	+	+	+
4	+	+	+
5	+	+	-
6	+	+	+
7	-	+	-
8	+	+	+
9	-	+	+
10	-	+	-
11	-	+	+
12	+	+	-
13	+	+	-
14	+	+	+
15	+	+	+

Investigation of the titer of Antistreptolysin O

This study showed that titer of Antistreptolysin O increased in the patients with tonsillitis caused by *S.pyogenes* , however the titer of 66.6% of the patients ASOT increased over (200-400) international unit / ml and 33.3% of the patients ASOT over (800-1600) international unit / ml Table (3), the increase in ASOT over (160-200) international unit / ml referred to recent infection by *S.pyogenes* (Jawetz *et al*., 1991). In conclusion: the ability of bacteria to cause disease has been attributed to have some virulence factors some of them play direct role and the other play indirect role and this factors cause injury in the host body.

Table 3. Titration of ASOT in serum of patients infected with tonsillitis

Global unit / ml titration of ASOT				number	group
1600-800		400-200			
percentage	number	percentage	number		
33.3	5	66.6	10	15	Total patients
40	4	60	6	10	acute
20	1	80	4	5	chronic

Streptococcus pyogenes plasmid profile

The plasmid –DNA content of one isolate has been detected, findings showed that isolate have one (large) plasmid band.

Plasmids curing

Acridin orange, ethidium bromide, sodium dodecyl sulfate have been used in order to cure plasmids of *Streptococcus pyogenes*. The result showed that the best concentration was 256 ug/ml by the using acridine orange, which able to cure plasmids from all isolates. The results was agreement (partially) with (Al-Saady *et al*., 2011), who found the best concentration was 512 ug/ml and the best concentration was 512 ug/ml by the using of ethidium bromide which able to cure plasmids from all isolates, while the concentration 2000 ug/ml has the ability to cure plasmids from all isolates by using sodium dodecyl sulfate. The results showed that the acrdine orange was the strongest curing material, while sodium dodecyl sulfate was the weaker material to cure plasmids Table (4).

Table 4. The results of curing plasmids

Concentration µg/ml	sodium dodecyl sulfate	ethidium bromide	acrdine orange
16	+++	+++	+++
32	+++	++	++
64	+++	++	+
128	++	+	+
256	++	+	+
512	++	+	-
1024	+	-	-
2000	+	-	-
2500	-	-	-
3000	-	-	-

Investigation of the sensitivity of isolates after and before curing

The results showed that some of isolates have been the ability to resistance antibiotics and become sensitive after curing of plasmids and this evidence that resistance to antibiotics exists on the plasmids, also some isolates sensitive after and before curing. Results showed that resistance to Ampicillin, Tetracycline, Trimethoprim, Erythromycin exists on the plasmids and the resistant isolates become sensitive after curing.

REFERENCES

Al-Baghdady, Isrra adnan ibraheam. 2006. Bacteriological and genetic studies on streptococci isolated from the upper respiratory tract infections. Thesis of master, Collage of Science, Babylon university.

Al-Saady, Lina Abd-Alameer salman. 2011. Bacteriological study of *pseudomonas aeruginosa* isolated from different clinical sources in Baqquba city and its suburbs. Thesis of master, collage of Education for pure science, Diyala university.

Angelescu, M. and Apostol, A. 2001. Cefepime (mexipime), large spectrum 4th generation cephalosporin, resistant to beta-lactamases. Chirurgia (Bucur). Nov-Dec. 96(6): 547-52 (Abstract).

Baldassarri, L, Creti, R, Recchia, S, Imperi, M, Facinelli, B, Giovanetti, E, Pataracchia, M. and Alfarone, G. 2006. Therapeutic failures of antibiotic used to treat Macrolide susceptible *Streptococcus pyogenes* infections may be due to Biofilm formation. *J of clinical microbiology*, 44(8): 2721-2727.

Bhardwaj, J.S, Angayarkanni, J, Bhattacharya, S, Das, A. and Palaniswamy, M. 2013. Isolation, screening and charctrization of beta-hemolytic Streptococci with potential of streptokinase production. *Int. Res. J. Biological sci*, 2(4):63-66.

Carapetis, J. R, Steer, A.C, Mulholland, E. and Weber, M. 2005. The global burden of Group A streptococci disease. Lanset Infect Dis, 5:685-694.

Cunnighaham, M.W. 2000. Pathogenesis of Group A streptococcal infections. Clin. Microbial. Rev, 13:470-511.

Doem, D.C, Roberts, L.A, Hong, W, Lukomski, S, Swords, E.W. and Reid, D.S. 2008. Bioflim formation by Group A Streptococcus: Arole for the Streptococcal regulator of virulence (Srv) and Streptococcal cysteine protease.

Goering, R.V.; Dockrell, H.M.; Wakelin, D.; Zuckerman, M.; Chiodini, P.L.; Roitt, I.M. and Mims, C. 2008. Mims medical microbiology. 4th ed. Mosby. China.

Jawetz, E, Melnick, J, L. and Adelberg, E.A. 1991. Medical Microbiology, 19thed prentice-hall. New Jersey. U.S.A.

kalin, H.E; 1999. The hole of β-lactam / β -Lactamase inhibitor in the management of mixed infections. *J.Antimicrobial. Agents. Chemoth.* 112 supp 111: 15-20.

Kayser, F. H.; Bienz, K. A.; Eckert, J. and Zinkernagel, R. M. 2005. Medical microbiology. 1st ed. Thieme Stuttgart, New York. U.S.A.

Macfaddin, J.F. 2000. Biochemical tests for identification of medical bacteria. "'3rdedition". The Williams and Wilkins. Baltimor, USA

Mojani, M, Ashtian, M.P. and Khanin, S.E. 2006. Plasmid associated lactocin PN78 production in alactobacillus adairy sample in Iran. *Med. J of Islamic World Academy of Science*, 16(1)19-24.

National Committee For Clinical Laboratory Standareds 2002. Perfomance Standared For Antibiotic Susceptibility Testing NCCLS. Villanova P.A.

Pagani, L.; Migliavacca, R.; Pallecchi, L.; Matti, C. and Giacobone, E. 2002. Emerging Extended-Spectrum B-Lactamases in *Proteus mirabilis*, *Journal of Clinical Microbiology, Apr*. p. 1549-1552

Razak, M.S. and Al-Jebori, R.F. 2012. Molecular study of storase enrzyme and characterization of some virulence factors in streptococcus pyogenes. *Med J of Babylon*, 9(1):74-83.

Riley, M. A, Goldstone, C.M, Wertz, J.E and Gordon. D. 2003. A phylogenetic approach to assessing the targets of microbial warfare. *J. Evol. Biol*, 16:690-697.

Sambrook, J.; E. F. Fritsch and T. Maniatis 1989. Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y.

Trevorse, J.T. 1986. Plasmid curing in bacteria. FEMS. *Microbiol. Rev*., 32: 149–157.

Wessels, M.R. 2006. Capsular polysaccharide of group A streptococci: inifischetti, Novick, R.P, Ferrrti, J.J, Portony, D.A. and Rood, J.I. Gram positive paythogenes. 2nded ASM press.

Whealer, M. C, Roe, M. H, Kaplan, F. I, Schlievert, P. M. and Todd, J. K. 1991. Outbreak of group A streptococci septicemia in children clinical, epidemiologic, and microbiological correlates. *JAMA*, 266: 533–537. (Abstract).

Zunino, P, Piccini, C and Fajardo, C.L. 1999. Growth: cellular differentiation and virulence factor expression by *Proteus miriabilis* in vitro and vivo. *J Med Microbial*, 48:527-534.

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