BioGecko
Vol 12 Issue 04 2023
ISSN NO: 2230-5807

Sensitivity of Staphylococcus Aureus to cephalothin and oxacillin in patients with secondary Sjogren's syndrome assessed by E-Test method

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Abstract

Purpose: Sjogren's syndrome is a systemic autoimmune disease that causes severe dry mouth leading to acute infection of parotid gland with methicillin resistant Staphylococcus Aureus (MRSA). Methicillin resistant antibiotics and first generation cephalosporins, both, have been suggested as the treatment of choice for this condition, yet there is no study that has compared the efficacy of these antibiotics to introduce the more effective one. The purpose of this study is to compare the sensitivity of Staphylococcus Aureus (SA) to cephalothin and oxacillin in patients with secondary Sjogren's syndrome by E-Test.

Material and Method: This study was conducted on 62 patients with secondary Sjogren's syndrome referred from a rheumatologist. Sampling was done from the right parotid gland and the samples were sent to the lab within 2 hours. After the microbial lab procedures (culture, isolation and SA identification), Minimum Inhibitory Concentration (MIC) was measured in each sample for both cephalothin and oxacillin by using E-Test. Positive samples for SA were collected and then coded. The data were analyzed by SPSS and Paired T-Test. We also compared MIC50 and MIC90 (MICs required to inhibit the growth of 50% and 90% of organisms, respectively) for these two antibiotics.

Result: SA was isolated from 30 patients (48%). The difference between the average MIC antibiotic sensitivity across these two antibiotics was significant (P=0.001). The correlation coefficient between variables was 0.821, which was significant (P<0.0001). MIC50 for oxacillin was higher than the value for cephalothin. The same was observed for MIC90.

Conclusion: The average MIC in oxacillin was greater than cephalothin. In other words, oxacillin was more effective than cephalothin.

Key words; Staphilococcus aureus, Sjogren's syndrome, E-Test

Introduction

Saliva has several functions in the upper gastro-intestinal tract, including antimicrobial, antacid, lubricative and coagulative functions. A disturbance in salivary function may lead to significant health problems. Sialadenitis, or inflammation of the salivary glands, has multiple causes. Sjogren's syndrome is an autoimmune disease affecting salivary glands through exocrinopathy shown in its physiopathology. Salivary glands involvement causes severe dry mouth leading to bacterial infection by Staphylococcus aureus (SA), mostly in the orifice of the parotid gland aurous(1). Several studies have shown that intra-venous methicillin resistant antibiotics or first generation cephalosporins could be the treatment of choice for this condition (1-10).

Methods:

This study was conducted on 62 patients with secondary Sjogren's syndrome referred from a rheumatologist. The disease was confirmed by the AECG criteria (1). Patients should not have received any antibiotic medication at the time of sampling or within 3 weeks before the study. Sampling was done from the right parotid gland by the paper points (numbers 50, 60 and 70 used according to anatomical differences in the salivary tract diameter), then each was inserted in glass tube of TSB as a transport media and sent to the microbiology laboratory in less than 2 hours.

Culture, Isolation and Identification of SA: The samples were cultured linear in the blood agar plates (Merk, Germany) enriched by 5% sheep blood and after 24 hours storage in incubator 35Ċ (Memmert,USA), the colonies being optimisted to Staphylococcus were analyzed by biochemical examinations with gram staining. Then the SA was identified by these tests respectively: Catalase, Manitol fermentation and Coagulase. Minimum Inhibitory Concentration (MIC), the minimum concentration of antibiotic that inhibits bacterial growth, was measured for each sample and each antibiotic by E-Test strips (MIC test strip, Liofilchem, Italy). This method has been suggested from CLSI as a standard procedure. By this method, initially a colony of the SA was inoculated in the Muler Hinton (Merk, Germany) broth agar and after 24 hours storage in 35Ċ, another suspension in 0.5 Mcfarland concentrations (equal to 1.5×108 bacteria/ml) was prepared. Then a grass culture of this concentration was done on the Muler Hinton agar. After water absorption in agar, a numerical E-Test strip was put on each plate and after 18-24 hours storage in 35Ċ, MIC in each sample was recorded by reading the effect on strip caused by surrounding bacterial hollow. According to the manufacturer instructions, the MIC measures lower than 2, were sensitive and greater than 4, were resistant to oxacillin, and measures between 2 to 4 were semi-sensitive. The values lower than 8, were sensitive and greater than 16 were resistant to cephalotin, and the valuestbetween 8 to 16 were semi-sensitive. MIC was measured for Standard ATCC25923 SA as a control. MIC 50 (the minimum inhibitory concentration in which half of the bacterial growth are sensitive to) and MIC 90 (minimum inhibitory concentration in which 90% of the bacterial are sensitive to) was recorded. After data collection and coding, we entered them to SPSS software and analyzed them by T-Test.

Results:

From 62 patients participated in this study, SA was isolated from 30 participants (48%) from whom 21 of them were female (70%) while 9 of them were male (30%).

The age range of the participants from whom the SA was isolted was between 55±12 years old while the total age range was 29 to 78 years old .In order to be 50% of the participant in each group, the age of 54 years, 11 months and 29 days of old was assumed as mid age in this study and the age range was divided into two group due to it.

The difference of average MIC between two antibiotics was 0.23±0.33 which was significant (p=0.001).(table1)

Table 1. Average MIC values in cephalothin across oxacillin

antibiotic	Average MIC	SD	N	P
oxacillin	0.43	0.36	30	0.001
cephalothin	0.56	0.67	30	

*MIC: Minimum Inhibitory Concentration

TheAverage MIC values in cephalothin and oxacillin in two age ranges has been shown in table 2.

Table2. Average MIC values in cephalothin across oxacillin in two age ranges

Age range	antibiotic	Average MIC	S.D	N
54-29	oxacillin	0.37	0.35	17
	cephalothin	0.59	0.53	17
78-55	oxacillin	0.5	0.38	13
	cephalothin	0.76	0.6	13

*MIC: Minimum Inhibitory Concentration

The correlation coefficient between variables was 0.821, which was significant ($p<0.0001$). Totally there was a significant relation between age and average MIC values ($p=0.004$ and $p=0.47$ for the age of 29-54 and 55-77, respectively).(table3)

Table 3. Difference of Average MIC values in cephalothin and oxacillin between two age ranges

Age range	Average MIC for cephalothin and oxacillin	SD	P
54-29	0.22	0.26	0
78-55	0.25	0.41	0.006

*MIC: Minimum Inhibitory Concentration

The Average MIC values in cephalothin across oxacillin in male and female has been shown in table4.

Table 4. Average MIC values in cephalothin across oxacillin in male and female

gender	antibiotic	Average MIC	SD	N
male	oxacillin	0.39	0.25	9
	caphalothin	0.58	0.22	9
female	Oxacillin	0.45	0.41	21

gender	antibiotic	Average MIC	SD	N
	cephalothin	0.71	0.67	21

*MIC: Minimum Inhibitory Concentration

Also there was a significant relationship between gender and average MIC (p=0.01 and p=0.008 for men and women, respectively).(table5)

Table5.Difference of Average MIC values for cephalothin and oxacillin in male across female

gender	Average MIC for cephalothin and oxacillin	SD	P
male	0.2	0.26	0
female	0.26	0.41	0.006

*MIC: Minimum Inhibitory Concentration

The results pertaining to MIC50 and MIC90 are separately presented for each age group and gender and the two antibiotics in Table 6.

Table6. MIC50 and MIC90 values across age and gender groups for oxacillin and cephalothin

	Age group				gender				total	
	55-78		29-54		female		male		antibiotic	
MIC	ox	ceph	ox	ceph	ox	ceph	ox	ceph	ox	ceph
MIC50	0.5	0.5	0.5	0.25	0.5	0.25	0.5	0.31	0.25	0.5
MIC90	0.2	0.11	0.25	0.17	0.25	0.13	0.39	0.13	0.13	0.25

*MIC: Minimum Inhibitory Concentration

Discussion:

Parotid glands only have serous secretions. A decrease or interruption of secretion can be caused by several factors that could be infectious (fungal, viral, and bacterial and…), or noninfectious (sialoliths, tumors, constrictions, drugs, anesthesia, autoimmunities, radiotherapies, allergy, diabetes, etc.). This in turn could results in decrease in ductal swish that leads to bacterial accumulation and growth around the Stenson's duct orifice in a retrograde manner and causes sialadenitis. Staphylococcus aureus is the most commonly identified bacterial species and the parotid gland has shown to be the most commonly involvement among other major salivary glands. Such an infection needs emergency care because of pain, swelling, and functional disabilities, extending in an acute and aggressive manner. Unless the

treatment had been done, Bell's palsy extending to the external auditory meatus, fistula formation and

mediastinitis would happen (11).

Nearly all of the authors have consensus on the antibiotic prescription beside supportive treatment (1-9,-11-17), but in Bradley et al. study (10), the incision and drainage has been suggested as a primary treatment.

As a treatment of choice it has been focused on the empiric antibiotic prescription of penicillins resistant to beta-lactamase or first generation cephalosporin. In some studies (12), vancomycin, ticolplanin and linezolid have been suggested if there was any resistant species and for non-responsive cases. According to Harbison et al. study (13), anaerobes and aerobes are possibly covered by clindamycin, cephoxitin, imipenem, metronidazole plus one macrolide or a penicillin plus clavulanate (Co-Amoxiclave).These results are the same as in Brook's study (12).

According to Enoch et al.(14), in two cases of bacterial sialadenitis with mixed bacterial involvement , after treatment with fluxacillin and metronidazole, vancomycin could cover the infection in resistant cases. As a conclusion, there is a consensus on the empiric antibiotic therapy on penicillins resistant to beta-lactamase and first generation cephalosporins. Brook (12) believes in MRSAs and gram negative species as a main cause in non-responding cases. There was a consensus in intravenous form of prescription (2, 12-14). Although diabetics, patients on anti-hypertensive and anti-depressant drugs have also dry mouth, in the current study we decided to sample from patients with Sjogren's Syndrome, since the dry mouth can be kproved by serum changes and biopsy as the diagnostic criteria of this syndrome according to AECG criteria, while in other diseases, collecting non-stimulating saliva is necessary which may not be accurate enough for definite diagnosis. Patients with secondary Sjogren's Syndrome are more frequent than primary ones, and we preferred them to take sample from.

According to one study (18) SA has been isolated from 55% of infections and remnant 45% of the cases are specified to: gram positive bacteria (Streptococcus viridans, pyogenic Streptococci, peptostreptococci, Hemophilus influenza and…) in 22% cases, gram negative bacillus (Pseudomona aeroginosa, Klebsiella pneumonia, Neisseria catharalis, E-coli) in 16% of cases and anaerobes (Salmonella, Fusobacterium, Bacteroides, Prevotella and Porphiromunas) in 4% of cases (10,12). In some other studies, parotiditis has been rarely reported from Mycobacterium Tuberculosis, Mycobacterium keluna and Mycobacterium fortutum (8-10).

E-Test method is the most sensitive and accurate method to evaluate microbial sensitivity in microbiology. It is also able to evaluate MIC. This method has more utility in resistant infections, parotiditis extended to anatomic spaces, and in hospitalized patients. Therefore, we used E-Test to compare two first line antibiotics to combat SA isolated from patients with Sjogren 's syndrome. In this study the patients should have not consumed any antibiotics at the time of sampling or within 21 days before it. In some studies this time has been considered to be 4 to 6 weeks, while in other studies the authors didn't persist on it but Chardin (21) excluded patients who had consumed antibiotics at the time of sampling and within 45 days before it.

The subjects' age range varied from 29 to78 years old and we divided them into two groups of 29-54 and 55-78 years-old. We found a significant difference between the bacterial sensitivity to oxacillin and cephalothin ($p=0.001$). The correlation coefficient between variables was 0.821, which was also significant ($p=0.001$) which shows linear relationship between the two groups and this means that every increase in oxacillin MIC value can be observed for cephalotin, too. There was a significant relationship between age and average MIC measurement for both antibiotics ($p=0.004$ and $p=0.47$ for age groups 29-54 and 55-78, respectively).

There was also a significant relationship between gender and average MIC measurement of both antibiotics.($p=0.01$ and $p=0.008$ for men and women, respectively).

As we compared MIC measure of the both antibiotics in two age groups, we observed that the amounts were higher in patients older than 55 years old; in other words, higher dosages is required to cover infection in elderly patients. We described such a difference from different aspects. First of all,

a young patient may have experienced fewer infections, less drugs and side effects of them like non responsiveness as a result of MRSA existence. But elderly patients have been suffered from dry mouth for many years, which leads to more DMFT values in two jaws. According to Mc Farlane study (22), these patients have shown greater isolation of SA, Candida albicans and Coliform bacillus. Secondly, poly-pharmacy is the main cause of dry mouth in elderly patients which can complicate xerostomia in a Sjogren's syndrome patient. As the third reason, it have been proved that xerostomia is more extensive and persistent among the elderly patient (1).

Observing greater average MIC values in women compared to men could be related to the factors affecting the local and systemic immunity. Recently, British scientists in Cambridge University have found micro-RNAs on the X-chromosomes being silent in the healthy situations and active during infection and neoplasia. This fact has also been supported by Belgian and Canadian scientists (23-25). In autoimmune disorders like Sjogren's Syndrome, these micro-RNAs cannot be as useful as they must be and weakens the systemic defense and raises the average MIC vale in in-vivo situations. In a study in China, researchers found greater values of DMFT, trauma from occlusion and wearing in women than men which reveals the weaker local immunity in women compared to men (26).

In the current study, we isolated SA from 30 patients (48%) which was more similar to the Jackson study in patients with RA (with 56%) than to Smith's in patients with Sjogren's (with 60%).. It may be because sampling by swap on mucosa can remove more bacteria than sampling by paper point. According to Smith et al. (27) SA can be isolated from 20% of healthy adults which is similar to Jackson et al. study (28) with 24% in adults, 36% in elderly patients and 56% in RA patients. This isolation would be greater in infants, elderly patients, RA and Sjogren's patients and blood dyscrasias. Again in Smith study, SA isolation was 23-48% in denture patients and 63% in denture patients with angular cheilitis.

SA is classified to MSSA (Methicillin Sensitive Staph Aureusaureus) and MRSA (Methicillin Resistant Staph Aureus). According to Smith (27) the MSSA varies depending on the region being sampled from. For example, sampling with swap from parotid duct in comparison with other regions of the mouth (tongue, buccal mucosa, floor of the mouth, and saliva) showed least isolated MSSA.

Resistance was reported in only 3% of the patients, and about 97% of the patients were MSSA. The MRSA prevalence is relates to age, hospitalization and missing teeth. The result of this study was similar to ours.

In a review study of Turnige et al. (20), from the studies of Fong et al.(29) and Regamey et al.(30), it has been concluded that the cephalosporins have a variable stability in front of betalactamase which relates to its chemical constructions.

According to the Steckleberg et al. (31) and Carrizosa et al. (32) , cephalotin and cephazolin were less effective in rabbit models with endocarditis. Glinka et al. (33), Vouillamoz et al. (34) and Chambers et al. (35), have concluded that the first generation cephalosporins are more effective than second and third generations. None of the of cephalosporins except cephamandol, cefuroxime and cefaclore, are effective against SA, but it is obvious that all cephalosporins can make primary coverage against it. Penicillins resistant to betalactamase (dicloxacillin, naficillin, oxacillin and flucloxacillin) are different from each other but this difference is not significant, and their average MIC has been calculated between 0.125 to 0.5.

Conclusion:

In the context of previous studies and based on the findings of the current study we can conclude that penicillins resistant to betalactamase could be the antibiotic of choice in the treatment of bacterial sialadenitis since they have broad spectrum and can cover both of the MSSA and MRSA.

Acknowledgements:
We would like to express our appreciation to Research Organization of Shahid Sadoughi University of Medical science of Yazd, Iran. We would like to thank them for the financial support. Special thanks are extended to Dr Hossein Soleymani for friendly compliance in patient referral. The results reported in this paper were part of a student thesis proposal.

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