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ANTIBIOTIC RESISTANCE AGAINST STAPHYLOCOCCAL ISOLATES RECOVERED FROM SUBCLINICAL MASTITIS IN THE NORTH OF PALESTINE

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مقاومة عزلات المكورات العنقودية من حيوانات مصابة بالتهاب الضرع دون السريري من شمال فلسطين لمجموعة من المضادات الحيوية

ملخص: أجريت هذه الدراسة لتحديد مدى مقاومة عزلات المكورات العنقودية لعشرة من المضادات الحيوية وذلك باستخدام طريقة انتشار القرص. لقد تم جمع هذه العزلات من عينات حليب ابقار، اغنام وماعز تعاني من التهاب الضرع دون السريري . كان مجموع هذه العزلات 132، حيث ان 66 عزلة تنتمي للمكورات العنقودية (*Staphylococcus aureus*) و أما ألمتبقية فهي تنتمي للمكورات العنقودية (*Staphylococcus epidermidis*). أثبتت النتائج ان 75.8% ، 66.7% من عزلات *S. aur*eus و *S. epidermidis* على التوالي كانت مقاومة للمضاد الحيوي أمبسلين (Ampicillin). المقاومة للمضادات الحيوية أميكاسين (Amikacin) ، سفيبيم (Cefepime) ، فانكومايسين (Vancomycin)، توبرامايسين (Tobramycin) والكلورامفينيكول (Chloramphenicol) كانت نادرة. وكما أثبتت الدراسة انه لا يوجد هناك أي عزلة من كلا المجموعتين حساسة لكافة المضادات الحيوية التي تم اجراء الفحص عليها. المقاومة لثلاث مضادات حيوية فما فوق كانت 53% للمكورات العنقودية *S .aureas* و 42.2% للمكورات العنقودية *S. epidermidis*.

Abstract: The antimicrobial resistance to 10 antibiotics was determined in 132 staphylococcal isolates. These representing *Staphylococcus aureus* (n=66) and *Staphylococcus epidermidis* (n=66). All isolates were from milk samples obtained from subclinical mastitis from Awassi ewes, local goats and Fresian cows. Results indicated that among all the antimicrobial agents tested the highest resistance of staphylococcal isolates was to ampicillin. The frequency of resistance to ampicillin was 75.8 and 66.7% against *S. aureus* and *S. epidermidis* isolates, respectively. Resistance to amikacin, cefepime, vancomycin, tobramycin or chloramphenicol was rare. None of staphylococcal isolates was susceptible to all tested antibiotics. Resistance to at least 3 drugs was found in (35) 53% and (28) 42.4 % of *S. aureus* and *S. epidermidis* isolates, respectively.

Key words: subclinical mastitis, antibiotic resistanse, multidrug resistance, *S. aureus*, *S. epidermidis*

Introduction

Staphylococci are the most prevalent and widespread species isolated from milk samples from subclinical mastitis [1-6]. The emergence of antibacterial resistance among microorganisms that impact domestic animal health has been a growing concern in veterinary medicine. Increased resistance of staphylococcal isolates recovered from mastitic domestic ruminants to different antimicrobial agents has been reported by several authors [7-9]. Antibiotic treatment of mastitis leads to significant increase in milk quantity and quality, lower somatic cell count and is likely associated with reduction in prevalence of clinical mastitis among herds, which is economically beneficial [10]. Bacterial identification and susceptibility tests are important for selecting the appropriate antimicrobial agent when treating mastitis [11]. Antimicrobial susceptibility of Staphylococci isolated from intramammary infected ruminants have been previously published [7-8, 11-23]. Drugs most commonly used are beta-lactams, aminoglycosides, macrolides, and lincosamides [24-25].

The purpose of this study was to determine the in vitro activity of selected antimicrobial agents against strains of *S. aureus* and *S. epidermidis* isolated from dairy domestic ruminants with subclinical mastitis in the north of Palestine during the period May to July of 2003.

Materials and Methods

A total of 132 Staphylococcal isolates were collected from raw milk samples of local goats (n=10), Awassi sheep (n=28) and Fresian cows (n=94) suffering from subclinical mastitis [6]. Milk samples were collected into sterilized screw cap bottles between May and July of 2003 and were immediately taken in a container containing ice cubes to the laboratory for bacteriological analysis.

Each milk sample (10µl) was surface plated on 5% sheep blood agar, nutrient agar and subsequently on mannitol salt agar. Samples were incubated at 37C for 24-48h under aerobic conditions. Gram stain and culture characteristics (colony morphology, pigmentation, and hemolysis) were used for presumptive identification for all isolates. Colonies suspected as staphylococcus were tested for coagulase test (tube method) as *S. epidermidis* is coagulase-negative and does not ferment mannitol. Further inoculations were made to confirm identification of the isolates biochemicaly.

The bacterial isolates were tested for their resistance using disk diffusion method [26]. Antibiotic disks (Oxoid) used were amikacin (30µg), ampicillin (10µg), cefepime (30µg), clindamycin (2µg), tetracycline (30µg),

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chloramphenicol (30µg), erythromycin (15µg), tobramycin (10µg), amoxicillin (10µg) and vancomycin (30µg). Triplicates of each antibiotic for each isolate were prepared. The diameter of inhibition zones were measured for each plate and the average reading of the three replicates for each antibiotic are shown in table 1. Zones of inhibition were determined in accordance with the National Committee for Clinical Laboratory Standard [27], isolates were categorized as susceptible and resistant while intermediate were considered as resistant.

Results

The antibiotic resistance profiles of the *S. aureus* and *S. epidermidis* isolates against 10 antimicrobial agents are presented in Table 1. Our results indicated that staphylococcal isolates in the North of Palestine exhibited high level of resistance to ampicillin with respect to all antimicrobial agents tested. The frequency of resistance to ampicillin was 75.8 and 66.7% against *S. aureus* and *S. epidermidis* isolates, respectively. Resistance to amikacin, cefepime, vancomycin, tobramycin or chloramphenicol was rare. None of staphylococcal isolates was susceptible to all tested antibiotics. Resistance to at least 3 drugs for both *S. aureus* and *S. epidermidis* is presented in Table 2a and 2b. It was found that (35) 53% and (28) 42.4 % of *S. aureus* and *S. epidermidis* isolates, respectively are resistant to at least 3 drugs.

Table 1. Antimicrobial resistant pattern of *S. aureus* and *S. epidermidis* isolates from sub-clinical mastitis.

The pathogens				
The antibiotics	*S. aureus* (n=66)		*S. epidermidis* (n=66)	
	Frequency	Percentage	Frequency	Percentage
Clindamycin	40	60.1%	35	53.3%
Tobramycin	7	10.8%	5	7.6%
Amikacin	4	6.1%	7	10.6%
Tetracycline	30	45.5%	27	40.9%
Chloramphenicol	1	1.5%	5	7.6%
Ampicillin	50	75.8%	44	66.7%
Vancomycin	5	7.6%	3	4.5%
Cefepime	3	4.5%	7	10.6%
Erythromycin	27	40.9%	23	34.8%
Amoxicillin	35	53%	26	39.4%

Table 2a. Pattern of multidrug resistance among *S. aureus* isolates.

Resistance patterns*	Frequency	Percentage
AMP-DA-AML-TE-E-TOB	5	7.6%
AMP-DA-AML-TE-E	14	21.2%
AMP-DA-AML-TE	9	13.6%
AMP-DA-AML	2	3.0%
AMP-DA-E	2	3.0%
AMP-E-TOB	1	1.5%
AMP-FEP-VA	1	1.5%
DA-AML-TE	1	1.5%

*AMP: Ampicillin; DA: Clindamycin; AM: Amoxicillin; TE: Tetracycline; E: Erythromycin; TOB: Tobramycin; C: Chloramphenicol; VA: Vancomycin.

Table 2a. Pattern of multidrug resistance among *S. epidermidis* isolates.

Resistance patterns*	Frequency	Percentage
AMP-DA-AML-TE-E-TOB	4	4.5%
AMP-DA-AML-TE	8	7.6%
AMP-DA-AML-C	3	6.1%
AMP-DA-TE-E	6	6.1%
AMP-DA-E	3	4.5%
AMP-E-AK	3	3.0%
AMP-DA-VA	1	1.5%

*AMP: Ampicillin; DA: Clindamycin; AM: Amoxicillin; TE: Tetracycline; E: Erythromycin; TOB: Tobramycin; C: Chloramphenicol; AK: Amikacin; VA: Vancomycin.

Discussion

Antibiotic resistant bacteria is a growing problem concern worldwide. Mastitis is the most common reason for antibiotic use in dairy herds. Effectiveness of current treatments and ability to control infectious diseases in both animals and humans may become in danger. Increased resistance of staphylococcal strains isolated from intramammary infections to several antimicrobial agents has been reported [7, 28]. In our study both isolates of *S. aureus* and *S. epidermidis* demonstrated high level of resistance to ampicillim. These results were consistent with a previous report from Poland, where 68.9% of *S. aureus* isolates were resistant to ampicillin [21]. However, these results were in contrast to other studies that reported lower resistance (7 to 49.6%) of *S. aureus* isolates recovered from mastitic domestic ruminants [12, 14, 17, 23].

Our data showed that our staphylococcal isolates are less resistant to tetracycline compared with that reported in a previous study [29] where it was found 69% of *S. aureus* isolates were resistant to this antibiotic. Other recent studies reported that 8.5 to 26% and 17.6 to 20.5% of *S. aureus* and Coagulase-negative staphylococcus isolates were resistant to tetracycline, respectively [2, 17, 21].

In the present study, staphylococcal isolates had high resistance to clindamycin. These data were in disagreement with that reported recently [19], where it was found that all isolates of *S. aureus* recovered from bovine mastitis were susceptible to this antibiotic. On the other hand, we found that most staphylococcal collection is sensitive to chloramphenicol and this is in accord with the same previous study [19], in which it was found that all isolates were susceptible to chloramphenicol. This may be due to that chloramphenicol is now used rarely for the treatment of domestic ruminants in this country.

In general our staphylococcal isolates showed high level of resistance particularly to ampicillin, clindamycin, tetracycline, amoxicillin and erythromycin which are commonly used in treatment of animals. This high rate of resistance to these antibiotics in our staphylococcal collection is likely due, in part, to selective pressure resulting from misuse of these antibiotics or commonly given antimicrobial growth promoters [30-32]. On the other hand, the behavior of amikacin, tobramycin, cefepime and vancomycin were proved to be the most active antimicrobials tested. This observation may provide the rational for alternative therapy that could be used in mastitic animals likely to be colonized with multidrug resistant staphylococcal isolates.

Resistance to at least 3 drugs was found in 53% and 42.4 % of *S. aureus* and *S. epidermidis* isolates, respectively. The presence of multidrug resistance in staphylococcal isolates has been reported by several investigators [11, 13, 33-35]. The emergence of multidrug resistant isolates may be due to misuse of antibiotics in animal production. The recommendation is for animals with staphylococcal mastitis to be culled from the dairy herd [8], because the appropriate therapy may become a serious clinical problem. For that reason, we can conclude that identification of mastitis pathogens is very important and antibiotic treatment is one of the recommended approaches in order to reduce intramammary infection and, consequently, the prevalence of mastitis in the herd. This could result in lower subclinical intramammary infection prevalence in the domestic ruminants. However, the indiscriminate use of antimicrobial agents either for treatment of mastitis or any other infection might generate an increase of the resistance level of many microrganisms to these drugs.

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