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Isolation and antibiotic resistance patterns of *Escherichia coli* and coagulase positive *Staphylococcus aureus* from honey bees digestive tract

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Summary

In this study antibiotic resistance pattern of *E. coli* and coagulase positive *Staphylococcus aureus* isolated from honey bees digestive tract of Shahrekord apiaries were studied. From 45 isolated *E. coli* strains 35 isolates (77.77%) were resistant to one or more antibiotics. The most resistance pattern were to Erythromycin (77.77%), followed by 14 isolates to Nitrofurantoin (31.11%). From 22 isolations *of S. aureus* resistance to Penicillin and Erythromycin were 18 (85.71%) and 11 (50.00%) isolates, respectively. Number of isolations in two seasons (fall and spring) was statistically significant ($P<0.005$), but profile of the antibiotic resistant of the two bacteria in two seasons are not statistically significant.

Key words: *E. coli, Staphylococcus aureus, A*ntibiotic resistance, Honeybee, Shahrekord

Introduction

Bacteria associated with bees are widely distributed in soil, water and air, stored bee food and surface of plants (Glinski and Jarosz, 1992).

Bee body coverings and the biochemical environment of the mid gut juice by bacteriostatic or bactericidal action effectively restrict the development of most infections caused by bacterial saprophytes (Jarosz, 1993).

The symbiotic microflora of the digestive tract of mature honey bee (*Apis mellifera*) consists of gram-negative, gram-positive and gram-variable bacteria, moulds and under some conditions also yeasts (Gilliam, 1987). The intestinal flora of the honey bee is susceptible to various chemotherapeutics and the species composition of this flora varies seasonally (Rada *et al*., 1997). The presence of S*taphylococci* and *E. coli* in the digestive tract of honey bee are well documented (Gilliam and Taber, 1991; Glinski and Jarosz, 1995; Rada *et al*., 1997).

Antibiotic resistance of bacteria is increasing worldwide and may result public health problems. Regarding the problem coagulase positive S*taphylococci* and *E. coli* have been subject of many studies (Maple *et al*., 1989; Tessi *et al*., 1997). However little is known about the antibiotic resistance profile of the coagulase positive S. *aureus* and *E. coli* with honey bee origin.

The Purpose of the present study was to isolate and examine antibiotic susceptibility of *S. aureus* and *E. coli* in the digestive tract of spring and fall honey bees of Shahrekord apiaries in central area of Iran.

Materials and Methods

Workers of *A. melifera* from 35 and 25 apiaries were collected in spring and fall of 2002, respectively. Five percent of hives in each apiary and 3-5 worker bees from each hive were taken randomly. Totally 393 hives (218 in spring and 175 in fall) were sampled. The workers were decapitated and their midgut and rectum were aseptically transferred into tubes (one tube for each hive) containing 5 ml sterile Triptose Soy Broth (TSB).

The tubes were placed at room temprature for 24 hrs. 0.1 ml aliquots of all tubes were plated on nutrient agar followed by24 hrs incubation at 37°C subculturing on

Eosin Methylen Blue (EMB) and manitol salt agar (MSA) and another incubation at 37°C for 24 hrs.

Suspected colonies of S*taphylococci* and *E. coli* were followed by standard microscopical and biochemical tests for isolation of coagulase positive *S. aureus* (catalase, coagulase, oxidase, o-f) and *E. coli* (lactose, IMVIC, lysin decarboxy lase, motility; Quinn *et al*., 2002).

Antibiotic resistance of the isolated organisms were determined by the disc diffusion method (Carter**,** 1973). Antibiotics tested were Gentamicin, Streptomycin, Kanamycin, Amikacin Penicillin, Chloramphenicol, Nalidixic acid, Oxytetracyclin, Erythromycin, Vancomycin and Nitrofurantoin prepared from Padtan Teb Co.

Differences in the number of the isolations and their antibiotic resistance profile among spring and fall bees were analyzed using chi-squared test.

Results

In the spring experiment, 33 *E. coli* and 15 isolates of coagulase positive *S. aureus* were confirmed. In the fall experiment, the number of isolates was 12 and 7, respectively. Differences between numbers of isolations in two seasons were statistically significant ($P<0.005$). While the differences in the resistance profile of the *E. coli* and *S. aureus* against antimicrobial agents in two seasons were not significant. Tables 1 and 2 show antibiotic resistance profiles of the *E. coli*and *S. aureus* in two seasons.

Table 1: Comparison of resistant and susceptible isolates to antimicrobial agents of honey bees *E. coli* in two seasons (NCCLS 1984)

Antibiotic	No of susceptibles (%)		No of resistants (%)	
	Spring	Fall	Spring	Fall
Kanamycin	3 (9.09%)	2 (16.66%)	14 (42.42%)	4 (33.33%)
Nalidixic acid	22 (66.66%)	10 (83.33%)	2 (6.06%)	1 (8.33%)
Erythromycin	0 (0.0%)	3 (25.00%)	30 (90.90%)	5 (41.66%)
Oxytetracyclin	13 (39.39%)	7 (58.33%)	8 (24.24%)	2 (16.66%)
Gentamicin	31 (93.93%)	12 (100%)	2 (6.06%)	0 (0.0%)
Amikacin	18 (54.54%)	12 (100%)	3 (9.09%)	0 (0.0%)
Streptomycin	22 (66.66%)	7 (58.33%)	5 (15.15%)	3 (25.00%)
Vancomycin	28 (84.84%)	4 (33.33%)	3 (9.09%)	5 (41.66%)
Chloramphenicol	27 (81.81%)	10 (83.33%)	0 (0.0%)	2 (16.66%)
Nitrofurantoin	10 (30.30%)	9 (75.00%)	12 (36.36%)	2 (16.66%)
Penicillin	1 (3.03%)	3 (25.00%)	10 (30.30%)	0 (0.0%)

Table 2: Comparison of resistant and susceptible isolates to antimicrobial agents of honey bees *S. aureus* in two seasons (NCCLS 1984)

Antibiotic	No of susceptibles (%)		No of resistants (%)	
	Spring	Fall	Spring	Fall
Kanamycin	5 (33.33%)	6 (85.71%)	6 (40.00%)	1 (14.28%)
Nalidixic acid	4 (26.66%)	3 (42.85%)	3 (20.0%)	4 (57.14%)
Erythromycin	4 (26.66%)	5 (71.42%)	11 (73.33%)	0 (0.0%)
Oxytetracyclin	8 (53.33%)	4 (57.11%)	2 (13.33%)	0 (0.0%)
Gentamicin	14 (93.33%)	7 (100%)	1 (6.66%)	0 (0.0%)
Amikacin	9 (60.00%)	7 (100%)	1 (6.66%)	0 (0.0%)
Streptomycin	12 (80.00%)	6 (85.71%)	1 (6.66%)	0 (0.0%)
Vancomycin	2 (13.33%)	5 (71.42%)	6 (40.00%)	2 (28.57%)
Chloramphenicol	15 (100%)	6 (85.71%)	0 (0.0%)	0 (0.0%)
Nitrofurantion	8 (53.33%)	6 (85.71%)	5 (33.33%)	0 (0.0%)
Penicillin	0 (0%)	0 (0.0%)	13 (86.66%)	5 (71.42%)

The *E. coli* isolates were resistant against Erythromycin (90.90% and 41.66%) in spring and fall samples while coagulase positive *S. aureus* isolates were resistant

against Penicillin (86.66%), Erythromycin (73.33%) in spring and to Penicillin (71.42%), Nalidixic acid (57.14%) in fall trials.

Discussion

Isolation of S*taphylocacci* and *E. coli* from the digestive tract of honey bees in this study is in agree with other investigations (Gilliam and Taber, 1991; Glinski, and Jarosz, 1995; Rada *et al*., 1997). Differences in the numbers of isolations in two seasons that are statistically significant ($P<0.005$) may be due to the differences in climatic conditions of spring and fall.

Bees as other insects are cold blood creatures and the environment of bee digestive tract does not provide optimal growth conditions for *E. coli* and *S. aureus* in fall season. Moreover the organisms may be more prevalent in the bee environment in spring season.

It seems that in cold seasons bee gut flora mostly composed of anaerobic bacteria in contrast to aerobic bacteria that are prevalent in warm seasons (Rada *et al*., 1997).

Higher resistance of *E. coli* and *S. aureus* isolates against Erythromycin (90.90% and 73.33% in spring) may be due to using of this antibiotic in Iran apiaries.

S. aureus isolates showed high resistance against Penicillin (86.66% and 71.42% in spring and fall respectively). Normaly this antibiotic is not used in apiaries, so high resistance of the organism may implies its human or animal origin.

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