International Scholars Journals

African Journal of Botany ISSN: 3519-3824 Vol. 8 (4), pp. 001-005, April, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Detection of the antibiotic resistance genes in *Staphylococcus aureus* isolated from human infections and bovine mastitis

Malihe Heidari[1]*, Hassan Momtaz[2] and Mahboobeh Madani[1]

[1]Department of Microbiology, Falavarjan Branch, Islamic Azad University, Falavarjan-Iran .
[2]Department of Microbiology, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord-Iran.
[3]Department of Microbiology, Falavarjan Branch, Islamic Azad University, Falavarjan-Iran.

Accepted 11 November, 2019

The present study was carried out in an attempt to detect the distribution of antibiotic-resistant genes of *Staphylococcus aureus* isolates from human infections and bovine mastitis. *mecA, msrA, msrB, aacA-D, tetK* and *tetM* genes were selected in order to detect the distribution of antibiotic-resistant genes by multiplex PCR technique. According to the biochemical analysis and detection of 16S-rDNA by PCR method, 108 isolates of 300 human infections samples and 18 strains from 150 bovine mastitis milks were recognized as *S. aureus*. Distributions of antibiotic-resistant genes in human isolates were as follows: (85.18%) *mecA*, (46.29%) *msrA*, (49.07%) *msrB*, (33.33%) *aacA-D*, (80.50%) *tetK* and (66.66%) *tetM* and in bovine mastitis, isolates were seen to be ranging from: (22.22%) *mecA*, (66.66%) *msrA*, (77.77%) *msrB*, (33.33%) *aacA-D*, (55.55%) *tetK* and (50.00%) *tetM*, respectively. Results indicated that all *S. aureus* strains have one or more of the antibiotic-resistant genes. Also, multiplex PCR technique is a fast, practical and appropriate technique for determining antibiotic-resistant genes. Hence, it was possible that the treatment and the right antibiotics were used.

Key words: Antibiotic-resistant genes, bovine mastitis, human infections, multiplex PCR, *Staphylococcus aureus*.

INTRODUCTION

Staphylococcus aureus is a Gram positive coccus (Steinberg et al., 1996) with circular chromosome located on those pathogenesis and antibiotic-resistant genes (Novick, 1990). It is one of the most important bacteria in Micrococcaceae family, which is also responsible for a wide variety of community- and hospital-acquired infections (Hiramatsu, 1998; Lowy, 1998; Abed EI-Jalil et al., 2008). This bacterium causes the pyogenic infections and toxigenic illness in humans and animals (Jahoda et al., 2007). *S. aureus* is one of the major causes of mastitis in cow, which is resistant against multiple antimicrobial drugs (Wang et al., 2008). Different strains of *S. aureus* and some of the coagulase negative staphylococci (CNS) species are agents of community-and hospital-acquired infections (Naimi et al., 2001). Moreover, *S. aureus* is the leading cause of bacterial infections involving the bloodstream, lower respiratory tract, skin and soft tissue in many developed countries (Herold et al., 1998; Gorak et al., 1999).

Inappropriate and the extra-usage of antibiotics have some roles in causing resistant *S. aureus* (Chambers, 1997; Hiramatsu, 1998). In 1940, some *S. aureus* strains showed resistance to penicillin. A decade later, multiple-resistant strains to tetracycline, chloramphenicol and erythromycin were reported (Kirby, 1944; Barber and Rozwadowska-Dowzenko, 1948). Methicillin-resistant *S. aureus* (MRSA) identified in 1960 was initially considered as a nosocomial pathogen (Vandenesch et al., 2006). *S. aureus* is also one of the major causes of mastitis in cow. However, mastitis treatment is not usually successful

*Corresponding author. E-mail: Heidarim_63@yahoo.com. Tel/Fax: 0098 381 3341365.

because this disease causes main damages in breast and drugs which are not able to make useful effects in all infection levels (Nickerson, 1993). On the other hand, this bacterium prevents phagocytosis and indirect cell-immunity (Yilmaz et al., 2007) and it also produces an enzyme which prevent most of the penicillin treatments (De Oliveira et al., 2000). It was also proven that beside methicillin antibiotic, *S. aureus* is resistant to other antibiotics like beta-lactam, amino-glycosides, fluoro-quinolones, lincosamides, macrolides and streptogramins (Chambers, 1987, 1997 Ramdani-Bouguessa et al., 2006), so it does not usually react to antibiotics treatment (Zecconi and Piccinini, 2002; Ochoa-Zarzosa et al., 2008; Wang et al., 2008; Kumar et al., 2010).

S. aureus strains diversify in antibiotic resistance by various mechanisms, such as modification of the ribosomal target site, enzymic inactivation of the drug, metabolic pathway alteration, efflux pumps and enzyme-tic cleavage of antibiotics (Schreckenberger et al., 2004; Yilmazet al., 2007; Ochoa-Zarzosa et al., 2008; Wang et al., 2008; Kumar et al., 2010). The antibiotic-resistant genes: *mecA* (methicillin), *aacA-D* (aminoglycosides), *tetK*, *tetM* (tetracyclines), *ermA*, *ermB*, *ermC* (macrolide–lincosamide–streptogramin B), *msrA* (macrolides) and *linA* (lincosamides) have been reported in last decade among the isolates of *S. aureus* (Ochoa-Zarzosa et al., 2008; Wang et al., 2008). *mecA* gene encodes *PBP2a*, *aacA-D* gene encodes bi-functional enzyme (Kumar et al., 2010), *msr* gene in staphylococci has caused resistance to macrolides and streptogramin B that is named as MSB phenotype and affects on efflux pumps activity (Merino-Díaz et al., 2007; Kumar et al., 2010). *erm(A,C)* gene causes modification of the bacteria's ribosome target site and encodes enzymes that by methylation of 23S rRNA causes reduction in connection of MLS_B antibiotic group to ribosome (Drinkovic et al., 2001; Młynarczyk et al., 2007; Kumar et al., 2010), and *tetK*, *tetM* for modification of the ribosome or effluxing (Kumar et al., 2010). Therefore, the detection of antibiotic-resistant genes in *S. aureus* strains with high virulent requires an effective guideline for the use of antibiotics (Ochoa-Zarzosa et al., 2008; Wang et al., 2008; Kumar et al., 2010).

Due to the shortage of information of antibiotic-resistant in *S. aureus* in Iran, this study was carried out in an attempt to detect antibiotic-resistant genes such as those resistant genes to methicillin (*mecA)*, macrolides (*msrA, msrB*), aminoglycosides (*aacA-D*) and tetracyclines (*tetK*, *tetM*) by the use of multiplex PCR method in *S. aureus* isolated from human infections and bovine mastitis.

MATERIALS AND METHODS

Detection of *S. aureus*

A total of 126 strains of *S. aureus* from various infections in human and bovine mastitis were examined. The samples taken from clinical materials (150 milk samples from cows and 300 samples of wound infections, urine infections, coetaneous abbesses and obstetric infections from human) were directly cultured onto 7% sheep blood agar and incubated aerobically at 37°C fo r 48 h. After incubation, suspicious colonies were examined by the use of morphologies compatible with *Staphylococcus* spp. and were transferred to Tryptic Soy Broth (TSB) (Merck) and Tryptic Soy Agar (TSA) (Merck). After growth, staphylococci were identified on the basis of colony characteristics, Gram staining, pigment production, hemolytic and the following biochemical reactions: catalyses activity, coagulated test (rabbit plasma), Oxidase test, O/F test with glucose, resistance to bacitracin (0.04 U), mannitol fermentation on Chapman agar, urease, nitrate reduction, novobiocin resistance, phosphatase, deoxyribonuclease (DNase) test, carbohydrate (xylose, sucrose, trehalose and maltose, fructose, lactose, mannose) fermentation tests (Zmantar et al., 2008; Kumar et al., 2010).

Amplification of antibiotic-resistant genes by multiplex PCR technique

The detection of antibiotic resistance genes by PCR was done using forward and reverses primers previously introduced by Kumar et al. (2010). PCR primers were chosen from the antibiotic resistance genes *mecA*, *aacA-D*, *tet K, tet M, msrA* and *msrB* and 16s-rDNA as listed in Table 1. *S. aureus* strains were grown on sheep blood agar plates overnight at 37°C. One colon y was suspended in 1 ml LB broth for 24 h at 37°C. Chromoso mal DNA was extracted by DNA Genomic Purification Kit (Fermentas, Germany). PCR was performed in a PCR thermocycler (Eppendrof Mastercycler, Eppendorf-Nethel-Hinz GmbH, Hamburg, and Germany).

The presence of the *mecA*, *aacA-D*, *tet K, tet M, msrA* and *msrB* genes encoding methicillin, aminoglycosides, tetracyclines and macrolides resistance was examined in 126 strains using multiplex PCR (Zmantar et al., 2008). Multiplex PCR assays were performed in 25 µl PCR mixtures 1 and 2. The mixture 1 contained 1 U of Taq DNA polymerase (Fermentas, Germany), 2.5 µl PCR buffer (10×), 1 µM each forward and reverse primers of *mecA*, *tet K* and *tet M* gene, 150 µmol/L of each dNTP and DNA template (50 ng). Using thermal cycling, the target genes were amplified (94° C 5 min, 30 cycles of 1 min at 95°C for the denaturation step and 1 min at 55°C for the annealing-extension step and 90 s at 72°C fo r the extension step). In mixture 2, the forward and reverse primers of the genes *aacA-D*, *msrA* and *msrB* (2.5 µM) were used. Ten microliters of PCR product was resolved on a 2% agarose gel containing 0.5 mg/ml of ethidium bromide in Tris–borate–EDTA buffer at 80 V for 1 h.

Statistical analysis

The data were analyzed using SPSS ver. 16.0 statistical software and a Chi-square test analysis was performed. Also, differences were considered significant at values of $p<0.05$.

RESULTS

Based on biochemical characteristics, from 450 tested samples, 126 isolates were identified as *S. aureus*. Out of 126 isolates, 108 had human and 18 strains were reported to have bovine origin. Amplification of 16s-rDNA confirmed all the 126 staphylococcal isolates as *S. aureus*. The distribution of *msrB* in human population was 49.07% and in bovine population was 77.77%. On

Table 1. Oligonucleotide primers for amplification of antibiotic resistance genes in *Staphylococcus aureus*.

Gene	Primer sequence	Size of product (bp)
mecA	F : AAAATCGATGGTAAAGGTTGGC R : AGTTCTGCAGTACCGGATTTGC	532
aacA-D	F : TAATCCAAGAGCAATAAGGGC R : GCCACACTATCATAACCACTA	227
tet K	F : GTAGCGACAATAGGTAATAGT R : GTAGTGACAATAAACCTCCTA	360
tet M	F : AGTGGAGCGATTACAGAA R : CATATGTCCTGGCGTGTCTA	158
msrA	F : GGCACAATAAGAGTGTTTAAAGG R : AAGTTATATCATGAATAGATTGTCCTGTT	940
msrB	F : TATGATATCCATAATAATTATCCAATC R : AAGTTATATCATGAATAGATTGTCCTGTT	595
16s-rDNA	F : GTAGGTGGCAAGCGTTACC R : CGCACATCAGCGTCAG	228

Table 2. Distribution of antibiotic-resistant genes in *Staphylococcus aureus* isolated of human infections and bovine mastitis.

Parameter	*mecA*(%)	*msrA*(%)	*msrB*(%)	*aacA-D*(%)	*tetK*(%)	*tetM*(%)
Human (n=108)	92 (85.18)	50 (46.29)	53 (49.07)	36 (33.33)	87 (80.50)	72 (66.66)
Bovine (n=18)	4 (22.22)	12 (66.66)	14 (77.77)	6 (33.33)	10 (55.55)	9 (50.00)
Total (n=126)	96 (76.19)	62 (49.20)	67 (53.17)	42 (33.33)	97 (76.98)	81 (64.28)

the other hand, the prevalence of *msrA* was considerably lower than the *msrB*. Only 50 isolates of human and 12 isolates of bovine origin yielded the band for *msrA*.

Among methicillin-resistant isolates, 96 (92 isolates of human and 4 isolates of bovine population) could amplify the *mecA* gene. Moreover, 33.33% of isolates were positive for *aacA-D*. The occurrence of *tetK* (80.50% in human and 55.55% in bovine isolates) among the bacterial isolates was significantly higher than *tetM* (66.66% in human and 50.00% in bovine population) gene. The details of distribution of antibiotic-resistant genes among the isolates are presented in Table 2. Besides, Pearson's Chi-square test revealed an asso-ciation that was significant (*P<0.05*) between *mecA* and *msrB* in bovine isolates and *mecA* and *aacA-D* in human isolates. Moreover, three strains (16.66%) were resistant to single antibiotic, while 5 strains (27.77%) showed resistance to 2 antimicrobial agents. Multi-resistance which is defined as resistance to 3 or more of drug tested was found in 55.55% of *S. aureus* strains isolated from bovine. In human isolates, 16 strains (14.81%) were resistant to single antibiotic and 22 strains (20.37%) showed resistance to 2 antimicrobial agents. Multi-resistance which is defined as resistance to 3 or more of drug tested was therefore found in 70 (64.81%) of *S. aureus* strains.

DISCUSSION

The present investigation was carried out to investigate the distribution of antibiotic-resistant genes including, *mecA, msrA, msrB, aacA-D, tetK* and *tetM* by multiplex PCR technique in *S. aureus* strains isolated from human infections and cow's milk. Multiplex PCR assays were successfully developed for the detection of six different resistance genes of genomic DNA of *S. aureus* isolates from human and bovine clinical samples. The results indicated that the prevalence of resistance to tetracycline was 76.98 and 64.28% for *tetK* and *tetM* genes, and tomethicillin and aminoglycosides were 76.19 and 33.3%, respectively. Also, the prevalence of gene resistance for

macrolides was 49.2 and 53.17% for *msrA* and *msrB* genes, respectively (Table 2).

The research by Vandensch et al. (2003) that was carried out on 117 CA-MRSA isolates from the United States, France, New Zealand and Western Samoa by PCR for 24 virulence factors, recognized resistant to the methicillin. In a survey by Turutoglu et al. (2006) on bovine mastitis, the antibiotic susceptibility test was carried out on 103 *S. aureus* and 136 coagulase-negative *staphylococcus* (CNS) strains, only 35 (10 *S. aureus* and 25 CNS) of the isolates were susceptible to all antibiotics being tested, while the remaining 204 isolates were resistant at least to one of the antibiotics. Among Staphylococci, 18 *S. aureus* strains and 31 CNS isolates were found phenol-typically resistant to penicillin G, ampicillin, amoxicillin and cloxacillin. Of 68 *S. aureus* isolates, 38 (55.9%) were β-lactam producers, and of the β-lactamase producer isolates, 21.1% were resistant to methicillin and remarkably 100% were susceptible to amoxicillin/clavulanic acid, while 97.4% were susceptible to ampicillin/sulbactam.

In addition, in the research carried out by Abed et al. (2008) on nasal carriage of MRSA, multiple- resistances to erythromycin, clindamycin and gentamicin were found in 48% of the MRSA. However, 38% of MRSA isolates were resistant to erythromycin and lincomycin but not to gentamicin and vancomycin. The resistant to other antibiotics was unusual in MRSA. In a study from Mexico, Ochoa-Zarzosa et al. (2008) reported that the distribution of antimicrobial-resistant genes encoding lincosamides, streptogramins and macrolides among bovine mastitis. Another study that was performed by Wang et al. in China (2008) has shown the resistant genes encoding lincosamides and macrolides in *S. aureus* isolates from bovine mastitis. In another similar study performed by Zmanter et al. (2008) on 35 *S. aureus* strains isolated from auricular infections by using of mPCR assay, the oxacillin resistance gene (*mecA*) and erythromycin genes (*ermA, ermB, ermC, msrA, msrB* and *mef*) were detected. The results showed that 60% of strains are *mecA* positive, while the frequency of erythromycin genes: *ermA* (22.8%), *ermB* (45.7%), *ermC* (17.1%), *msrA* (28.6%) and *mef* gene was not detected in any *S. aureus* strain. The research carried out by Kumar et al., (2010) has shown that *S. aureus* is one of the major causes of mastitis in dairy animals and its resistant against multiple antimicrobials always remain of crucial concern.

The 126 strains of *S. aureus* isolated from bovine mastitis were collected and tested for antibiotics with disc-diffusion method. Besides, resistant genes *linA, vatA, vatB, vatC, msrA, msrB, ermA, ermC, aacA-D, tetK, tetM* and *mecA* were detected by PCR. The phenotypic antibiotics resistance percent in *S. aureus* isolates was classified as gentamicin (30.5%), kanamycin (25.8%), tetracycline (36.7%), penicillin G (22.7%) and strepto-
mycin (26.6%). All isolates were susceptible to vancomycin. The distribution of antibiotic-resistant genes was *linA* (51.6%) followed by *msrB* (46.1%), *tetK+M* (34.4%), *msrA* and *aacA-D* (26.6%). Different antibiotic-resistant genes combinations were presented. All isolates lacked amplification of *vatA, vatB, ermA* and *ermC* genes, which showed significant prevalence of resistant to multiple antibiotics. The results of the present investigation, just as other researches done in the different parts of the world, are representatives of significant of *S. aureus* strains that are resistant to the antibiotic in level of animal and human populations, which could arise due to unlimited consuming of antibiotic in the society and animal treating centers.

Multiplex PCR technique is a very fast and inexpensive technique for detecting antibiotic resistant genes in resistant strains. It is required to control the transfer or spread of pathogenic strains and from separate cows having mastitis in order to prevent the transfer of infection from one cow to another. Furthermore, antibiotic susceptibility tests should be done besides detecting bacterial factors in order to enhance treatments for decreasing infections of human staphylococci and bovine mastitis.

ACKNOWLEDGEMENTS

The authors thank Mr. M. Momeni, D. Rahimian and S. Safari at the Biotechnology Research Center and Microbiology laboratory of the Islamic Azad University of Shahrekord, for their sincere technical support. This work was supported by Grant No. 68565 from the Islamic Azad University of Shahrekord Branch in Iran.

REFERENCES

Novick RP (1990). The *staphylococcus* as a molecular genetic system in Molecular biology of the staphylococci. New York VCH.

Abed El-Jalil H, Jallad M, Thwaini AJ (2008). Nasal Carriage of methicillin resistant *Staphylococcus aureus* in individuals exposed and not exposed to hospital environments. Eur. J. Sci. Res., 22: 570-574.

Barber M, Rozwadowska-Dowzenko M (1948). Infection by penicillin-resistant staphylococci. Lancet 2: 641-644.

Chambers HF (1987). Coagulase-negative staphylococci resistant to beta-lactam antibiotics *in vivo* produce penicillin-binding protein 2a. Antimicrob. Agents Chemother., 31: 1919-1924.

Chambers HF (1997). Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Clin. Microbiol. Rev., 10:781-791.

De Oliveira AP, Watts JL, Salmon SA, Aarestrup FM (2000). Antimicrobial susceptibility of *Staphylococcus aureus* isolated from bovine mastitis in Europe and the United States. J. Dairy Sci., 83: 855-862.

Drinkovic D, Fuller ER, Shore KP, Holland DJ, Ellis-Pegler R (2001). Clindamycin treatment of *Staphylococcus aureus* expressing inducible clindamycin resistance. J. Antimicrob. Chemother., 48: 315-316.

Gorak EJ, Yamada SM, Brown JD (1999). Community-acquired methicillin-resistant *Staphylococcus aureus* in hospitalized adults and children without known risk factors. Clin. Infect. Dis., 29: 797-800.

Herold BC, Immergluck LC, Maranan MC, Lauderdale DS, Gaskin RE, Boyle-Vavra S, Leitch CD, Daum RS (1998). Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. JAMA., 279: 593-598.

Hiramatsu K (1998). The emergence of *Staphylococcus aureus* with reduced susceptibility to vancomycin in Japan. Am. J. Med., 104: 7S-

10S.
Jahoda D, Nyc O, Pokorný D, Landor I, Kr ůta T, Sosna A (2007). Treatment of orthopedic infections caused by resistant staphylococci. Folia Microbiol., (Praha) 52: 281-285.
Kirby WM (1944). Extraction of a highly potent penicillin inactivator from penicillin resistant staphylococci. Sciences, 99: 452-453.
Kumar R, Yadav BR, Singh RS (2010). Genetic determinants of antibiotic resistance in *Staphylococcus aureus* isolates from milk of mastitic crossbred cattle. Curr. Microbiol., 60: 379-386.
Lowy FD (1998). *Staphylococcus aureus* infections. N. Engl. J. Med., 339: 520-532.
Merino-Díaz L, Cantos de la Casa A, Torres-Sánchez MJ, Aznar-Martín J (2007). Detection of inducible resistance to clindamycin in cutaneous isolates of *Staphylococcus* spp. by phenotypic and genotypic methods. Enferm. Infecc. Microbiol. Clin., 25: 77-81.
Młynarczyk G, Młynarczyk A, Szymanek K, Bilewska A, Luczak M (2007). The correlation between phenotypes and genotypes of macrolides, lincosamides and streptogramins B resistance in *Staphylococcus aureus*. Med. Dosw. Mikrobiol., 59: 17-25.
Naimi TS, LeDell KH, Boxrud DJ, Groom AV, Steward CD, Johnson SK, Besser JM, O'Boyle C, Danila RN, Cheek JE, Osterholm MT, Moore KA, Smith KE (2001). Epidemiology and clonality of community-acquired methicillin-resistant *Staphylococcus aureus* in Minnesota, 1996-1998. Clin. Infect. Dis., 33: 990-996.
Nickerson PC (1993). Eliminating chronic staphylococcal mastitis. Vet. Med. 88: 375-381.
Ochoa-Zarzosa A, Loeza-Lara PD, Torres-Rodríguez F, Loeza-Angeles H, Mascot-Chiquito N, Sánchez-Baca S, López-Meza JE (2008). Antimicrobial susceptibility and invasive ability of *Staphylococcus aureus* isolates from mastitis from dairy backyard systems. Antonie Van Leeuwenhoek, 94: 199-206.
Ramdani-Bouguessa N, Bes M, Meugnier H, Forey F, Reverdy ME, Lina G, Vandenesch F, Tazir M, Etienne J (2006). Detection of methicillin-resistant *Staphylococcus aureus* strains resistant to multiple antibiotics and carrying the Panton-Valentine leukocidin genes in an Algiers hospital. Antimicrob. Agents Chemother., 50: 1083-1085.
Schreckenberger PC, Ilendo E, Ristow KL (2004). Incidence of constitutive and inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci in a community and a tertiary care hospital. J. Clin. Microbiol., 42: 2777-2779.
Steinberg JP, Clark CC, Hackman BO (1996). Nosocomial and community-acquired *Staphylococcus aureus* bacteremias from 1980 to 1993: impact of intravascular devices and methicillin resistance. Clin. Infect. Dis., 23: 255-259.
Turutoglu H, Ercelik S, Ozturk D (2006). Antibiotic resistance of *Staphylococcus aureus* and coagulase negative staphylococci isolated from bovine mastitis. Bull. Vet. Inst. Pulawy, 50: 41-45.
Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, Liassine N, Bes M, Greenland T, Reverdy ME, Etienne J (2006). Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg. Infect. Dis., 9: 978-984.
Wang Y, Wu CM, Lu LM, Ren GW, Cao XY, Shen JZ (2008). Macrolide-lincosamide-resistant phenotypes and genotypes of *Staphylococcus aureus* isolated from bovine clinical mastitis. Vet. Microbiol., 130: 118-125.
Yilmaz G, Aydin K, Iskender S, Caylan R, Koksal I (2007). Detection and prevalence of inducible clindamycin resistance in staphylococci. J. Med. Microbiol., 56: 342-345.
Zecconi A, Piccinini R (2002). Control of Staphylococcus aureus infection - practical aspects and perspectives. Practical aspects of scientific examinations of reproduction system and mammary gland in animals. Wenecja. Wyd. PlWet. Puławy, 18-30.
Zmantar T, Chaieb K, Ben Abdallah F, Ben Kahla-Nakbi A, Ben Hassen A, Mahdouani K, Bakhrouf A (2008). Multiplex PCR detection of the antibiotic resistance genes in *Staphylococcus aureus* strains isolated from auricular infections. Folia Microbiol., (Praha), 53: 357-362.