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Molecular Screening of Antibiotic-Resistance Gene of Bacteria Isolated from Mastitic Cow

By

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Summary

VI. SUMMARY

A total of 415 lactating Holstein cows from different localities in Delta area, Egypt including 345 cows from four dairy farms at Damietta (farm A, 60 lactating cows, farm B, 65 lactating cows and farm C, 120 lactating cows) and El-Sharkia (farm D, 100 lactating cows) Governorates in addition to 70 individual cases of dairy cows at El-Dakahlia Governorate; were examined for clinical and subclinical mastitis during the period from October 2014 to June 2018.

Concerning to the prevalence rate of clinical and subclinical mastitis, it was found that the overall prevalence of mastitis in this study was 224 (54%) at the cattle level, 50 (12%) were clinical and 174 (42%) were subclinical cases. The prevalence rate at the quarter level was 467 (52.1%), 106 (11.8%) were clinical and 361 (40.3%) were subclinical.

Regarding to quarter involvement, the affection of two quarters was higher than the other quarters in clinical and subclinical mastitic cows with prevalence rate of 38% for clinical cases and 44.25% for subclinical cases, respectively. In clinically mastitic cows, one-quarter affection was 34%, three quarters affection was 10% and four quarters affection was 18% while, in subclinical cases one quarter affection was 27.01%, three quarters affection was 22.9% and four quarters affection was 5.7%. Furthermore, hindquarters' affection was more prevalent than forequarters in both clinical and subclinical cases in our study.

All the potential risk factors considered in the present study namely, season of the year, hygienic measures and age of the cow affected prevalence of mastitis.

Regarding to the effect of season of the year on the prevalence of mastitis, clinical mastitis in the examined dairy cattle was higher in spring (23.5%) and winter (21.4%) than in autumn (6.12%) and no clinical cases were found during summer. Furthermore, subclinical mastitis was higher in winter (53.8%) and spring (47%) than summer (37.5%) and autumn (34.7%).

Concerning hygiene, dairy cows with good hygienic measures had a low prevalence of mastitis at a rate 1.6% for clinical mastitis and 31.35% for subclinical

mastitis. However, dairy cows with bad hygiene had a high prevalence of mastitis at a rate of 20.4% for clinical mastitis and 50.4% for subclinical form.

In relation to age of cows, the present study revealed that animals in (≥ 5-9 years) group were more susceptible to clinical mastitis (13.5%) than those in age group (≥ 2-4 years) (11.02%). In addition, old cows (≥ 5-9 years) were more susceptible to subclinical mastitis (43.5%) than young cows (≥ 2-4 years) (40.8%).

From etiological point of view, the current bacteriological examinations revealed that CNS were the predominant bacteria isolated from mastitic cases (32%) followed by, *E. coli* (24.8%), *S. aureus* (15.4%), *Str. agalactiae* (13.4%), *Str. dysgalactiae* (8.8%), *E. faecalis* (2%), *Str. uberis* (2%), *Enterobacter aerogenes* (0.66%), *Micrococcus* (0.66%), *Enterobacter agglomerans* (0.22%) and *Klebsiella* spp. (0.22%).

In clinical mastitis, there were *S. aureus* (14.3%), CNS (25.7%), *Str. agalactiae* (14.3%), *Str. dysgalactiae* (8.6%), *Str. uberis* (2.86%), *E. faecalis* (2.86%), *E. coli* (24.7%), *Klebsiella* spp. (0.95%), *Enterobacter aerogenes* (2.86%), *Enterobacter agglomerans* (0.95%) and *Micrococcus* (1.9%).

In subclinical mastitis, there were *S. aureus* (15.7%), CNS (34 %), *Str. agalactiae* (13%), *Str. dysgalactiae* (8.8%), *Str. uberis* (1.7 %), *E. faecalis* (1.7 %), *E. coli* (24.8%) and *Micrococcus* (0.3 %).

In the present study, the results of antibiotic sensitivity test for Staphylococcus spp. revealed that oxacillin was the most resistant antibiotic (92%), followed by ampicillin (90%), cefoxitin (86%), cefotaxime (70%), tetracycline (70%), ampicillin/sulbactam (64%), sulphamethoxazole/trimethoprim (64%), erythromycin (58%), gentamicin (50%), ofloxacin (42%), chloramphenicol (40%), ciprofloxacin (34%) and vancomycin (2%).

Regarding to the antibiogram of *S. aureus* isolates using disc-diffusion method revealed that they were highly resistant to oxacillin (96.6%) followed by ampicillin (93.3%), cefoxitin (93.3%), tetracycline (73.3%), cefotaxime (70%), ampicillin/sulbactam (66.67%), erythromycin (56.6%),

sulphamethoxazole/trimethoprim (56.6%), gentamicin (53.3%), ofloxacin (40%), chloramphenicol (36.6%), ciprofloxacin (30%) and vancomycin (0%).

Results of antibiogram of CNS isolates revealed that the highest number of isolates were resistant to ampicillin and oxacillin (85%, each), followed by cefoxitin (75%), sulphamethoxazole/ trimethoprim (75%), cefotaxime (70%), tetracycline (65%), ampicillin/sulbactam (60%), erythromycin (60%), chloramphenicol (45%), gentamicin (45%), ofloxacin (45%), ciprofloxacin (40%) and vancomycin (5%).

Concerning results of antibiotic sensitivity using VITEK 2 compact, it was found that *S. aureus* isolates were highly resistant to penicillin, cefoxitin, oxacillin with percentage (100%, each), followed by tetracycline (54%), erythromycin and clindamycin (30.7%, each), gentamicin (15.4%) and quinopristin/dalfopristin (7.7%).

In the present study, all of the examined Staphylococci isolates were resistant to at least three antibiotics resulting in high MAR index which was more than 0.2 where ten isolates were resistant to 10 antibiotics (20%), seven isolates were resistant to 9 antibiotics (14%), six isolates were resistant to 5 antibiotics (12%), six isolates were resistant to 6 antibiotics (12%), five isolates were resistant to 4 antibiotics (10%), four isolates were resistant to 11 antibiotics (8%), three isolates were resistant to 8 antibiotics (6%), two isolates were resistant to 3 antibiotics (4%) and two isolates were resistant to 12 antibiotics (4%).

Concerning antimicrobial phenotypic profiles, the most exhibited phenotype was Oxacillin – Ampicillin - Cefoxitin (OX-AM- FOX) in both *S. aureus* and CNS indicating high levels of resistance to these antibiotics.

In respect to using PCR for confirmation of Staphylococcal infections, the nuc gene and coa genes were used for confirmation of the isolated *S. aureus*. Results revealed that all the recovered *S. aureus* harbored the amplified products of both genes. On the other hand, the tuf gene was used for confirmation of CNS species where all of them were positive for this gene indicating high correlation between biochemical identification and genetic detection of these isolates.

Regarding to the prevalence of antibiotic resistance genes of Staphylococci spp., the obtained results revealed that all of the 25 Staphylococcal isolates were found to be

positive for the presence of mecA (100%) and 24/25 isolates were positive for the presence of blaZ gene (96%) where 19/25 isolates were phenotypically resistant to β-lactam antibiotics, 3/25 expressed intermediate resistant phenotype for ampicillin and also, 3/25 were intermediate resistant for oxacillin. Detection of tetK gene in 17/25 isolates (68%) where 15/17 were resistant phenotypically to tetracycline, one isolate was intermediate resistant and one isolate was sensitive to tetracycline although carrying tetK gene. Only 6/25 isolates were positive for the presence of fexA gene (24%) where three isolates were phenotypically resistant to chloramphenicol, one isolate was intermediate resistant and two isolates were sensitive although carrying fexA gene.