

Studies on the *Pseudomonas aeruginosa* isolated from fleece rot in sheep suffering from dermatitis in northern & middle Jordan

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Abstract

A total of 162 sheep fleece samples (7%) were bacteriologically examined. Seventy nine sheep of them showed clinical signs in which exudation and abcessation were recorded in 21 (13%) sheep, while fleece coloration was observed in 18 (11 %) and mixed clinical signs in 7 (4 %). The rest 83 (51 %) sheep showed no obvious clinical signs. Seventeen *Pseudomonas aeruginosa* isolates were cumulated. The isolates were identified biochemically and their susceptibility toward 16 commonly used antibiotics was determined. The resistance toward amikacin, ciprofloxacin and the norfloxacin was determined in 11.7%, 23.4 % and 29.4%, respectively while the knanmycin, streptomycin, tetracycline, amoxycillin, erythromycin and the co-trimoxazol were ineffective against more than 90% of the *P. aeruginosa* isolates. Intermediate resistance against each of the norfloxacin, ciprofloxacin, enrofloxacin, kanamycin, streptomycin, tetracyclin, amocycillin, erythromycin, ampicillin, neomycin, doxycyclin, nalidixic acid, amikacin, nitrofuantoin was recorded among 6, 7, 5, 5, 2, 1, 1, 1, 2, 3, 7, 1 and 2 of the examined *P. aeruginosa* isolates.

Fourteen (82%) isolates were recorded as serum resistant and their count after 1-3h incubation in the sheep and calf sera, increased by 2-2.9 and 2.5-3.5 logs, respectively.

Introduction

Sheep fleece rot is an exudative bacterial dermatitis associated and influenced by the low hygienic raising conditions such as prolonged wetting of the skin (2, 8). *P. aeruginosa* seems to be the main causative organism of fleece rot and it was concluded that it contributes in the disease complex. The importance of *P. aeruginosa* which was associated with increased severity of fleece rot and subsequent flystrike can not be underestimated and warrants consideration for inclusion it in fleece rot diagnosis and control trials such as vaccination (8). Experimentally, the sheep which were inoculated cutaneously with *P. aeruginosa* developed rapidly a green coloration while the animals which were kept dry, showed no signs of dermatitis (2). This coloration was associated with an outpouring of serous exudates in the fleece rot lesion and with the formation of microabscesses (2).

On the other side, although the *P. aeruginosa* was evaluated as possible cause, in part, to the disease complex, its role and severity in the fleece rot should not be underestimated (8). It should also be taken in consideration in the diagnosis of the fleece rot as well as in its control trials e.g. vaccination (8).

Serologically, the sheep which were wetted and inoculated with *P. aeruginosa*, unlike the dry animals, showed a serologic reactivity (2). Such seroreactivity was against the outer rather than the inner membranous protein of the bacterium (2).

P. aeruginosa is among the bacteria, which show a remarkable resistance toward many antibiotics (1, 5). Clinically, significant *P. aeruginosa* strains were found resistant to eight relatively new antibiotics (1). Such a resistance ranged from 9.1% to 31.9% toward the individual antibiotics (1). Combination of two or more antibiotics is likely to be needed to assign the antibiotic treatment protocol. For example, combinations of the gentamicin, lincomycin, spectinomycin and tylosin or the combination of penicillin, streptomycin, lincomycin, spectinomycin and minocycline were tried but yet *P. aeruginosa* recovered by *in-vitro* testing of such combination (5, 6).

Serum-resistance which counteracts the bactericidal effect of the complement system is an important factor in the pathogenicity of *P. aeruginosa* (12). Thirty seven percent of clinical isolates were found to be serum resistant (13). However, such a resistance was attributed to certain serotypes (12) where the serotyping, along with the isolation site, antibiotic resistance, and beta-lactamase production were approached to determine the profiles of *P. aeruginosa* from clinical isolates (7, 14, 12).

Since, in Jordan, no information is available about this affection, this study aims to determine the prevalence of fleece rot dermatitis among sheep herds in the northern and middle sectors of Jordan, to isolate the bacterial causation with special reference to the *Ps. aeruginosa* and to determine the antibiotic sensitivity and the serum resistance patterns of the isolates

Materials and Methods

A total of 162 wool samples were aseptically collected from 32 sheep herds (2273 heads). Collection of the samples was reconsidered, as possible, to be from animals showing abnormal skin problems especially from those having skin/wool discoloration or associated with an exudates and abscesses.

The samples were processed, promptly. They were cultivated on different enriching / differential media, namely: *Pseudomonas* selective agar, Sheep Blood Agar, MacConkey Agar, Tryptic Soy Agar and Tryptic Soy Broth. All of the media were from OXOID. The media were incubated at 37°C for 24-48 h under aerobic conditions.

A total of 17 (10.5%) isolates showing the general characteristics of the *Pseudomonas aeruginosa*, specially the production of greenish/bluish pigmentation and/or the characteristic fruity odor, were cumulated.

For confirmatory diagnosis and for characterization the isolates, different biochemical reactions were determined, namely: oxidase production, growth at low (5°C) and high (42°C) temperature, citrate utilization, OF (glucose fermentation), sugar fermentation (fructose, mannitol and xylose), nitrate reduction, gelatin liquefaction, urease production, tween hydrolysis and the motility reactions according to Koneman (9) and Cowan & Steel (4). Sensitivity to antibiotics was conducted by determination of the minimum inhibitory concentration in solid medium according to the guidelines of the National Committee for Clinical Laboratory Standards (11).

For determination of their serum resistance, blood of 6 healthy sheep as well as of 3 healthy young calves was collected and the serum of each species was pooled. To assay (15) the serum resistance of the *P. aeruginosa* isolates, 80% of each untreated and heat treated (56°C/30 min) serum was used. The serum heating aims to inactivating the complement bactericidal components. An 18h old tryptic soy broth culture of each isolate was subcultured into a tryptic soy broth and was grown to the mid-log

phase. Cells (approximately 3×10^7 / ml) were added directly to treated and untreated sera and incubated at 37°C for certain intervals. After elapsing of 0, 1, 2 and 3 hours, the viable bacterial count was determined, in triplicate, by ten folds dilution (15).

Results

A total of 162 samples (7%) were examined (Table 1) from which, 17 *P. aeruginosa* isolates were cumulated. The samples were collected almost equally from both sexes but the majority (127 samples) were from elder sheep (1-2 years old). Clinical signs could be observed in 79 sheep. The exudation and abcessation were recorded in 21 (13%), the coloration in 18 (11 %) and mixed clinical findings were recorded in 7 (4 %). The majority, 83 (51 %) of the examined sheep showed no obvious indicative clinical signs.

The antibiotic resistance patterns of the 17 *P. aeruginosa* isolates against 16 antibiotics are displayed in Table 2. The recent antibiotics like the amikacin, ciprofloxacin and the norfloxacin were not fully effective where resistance against them was determined in 11.7%, 23.4 % and 29.4%, respectively. On the other side, the antibiotics of old generation like the knanmycin, streptomycin, tetracycline, amoxycillin, erythromycin and the co-trimoxazol which were ineffective against more than 90% of the tested *P.aeruginpsa* examined strains. However, intermediate resistance against each of the norfloxacin, ciprofloxacin, enrofloxacin, kanamycin, streptomycin, tetracyclin, amocycillin, erythromycin, ampicillin, neomycin, doxycyclin, nalidixic acid, amikacin, nitrofuantoin was recorded among 6, 7, 5, 5, 2, 1, 1, 1, 2, 3, 7, 1 and 2 of the examined *P. aeruginosa* isolates.

Figures 1 and 2, show the serum resistance patterns of the 17 *P. aeruginosa* strains. By using the heated (inactivated) sheep serum (Fig. 1), the count of all 17 isolates increased by 2 - 3.1 logs while the count only of 3 isolates incubated in the untreated serum decreased by 0.7 - 1.3 logs. The other 14 (82%) strains were recorded as resistant and their count increased in 2-2.9 logs. By using the calf serum, almost the same pattern among the 17 isolates was recorded. However, the same previously notified 14 (82%) resistant strains showed, relatively, higher count which was ranging from 2.5-3.5 logs (Fig. 2) while the count of sensitive ones decreased in 1-1.8 logs.

Discussion

Wool production is a strategic target of sheep raising. Certain factors may predispose the skin affection which results to dermatitis and consequently to lowering of the fleece quality (2, 8). In this study, about 49% of the examined sheep showed various skin/fleece denaturations (table 1) from which about 13% were having clinical signs in form of exuadation and abcessation. The incidence of fleece rot increases in humid environment and/or in wet skin (2, 8) especially among the unvaccinated (8) herds. In this study, the *P. aeruginosa* was isolated in 10.5% from the examined samples which indicates its remarkable role (2) in sheep dermatitis and subsequently in the development of fleece rot in Jordan. This is strongly predicted, especially that 13 out of the 17 (82%) isolates were cumulated in the period between December-April months which are wet and/or humid months in Jordan (2, 8). Indeed, in March-April months in which the atmospheric temperature starts to increase, 6 (35%) isolates were cumulated and the role of flies in spreading of the infection among herds (8) can not be excluded.

In Jordan, lack of vaccination attempts (8) for competing such affections, may add some other factor in this sheep health problem. On the other hand, unlike the sex, the age can be counted as another predisposing factor since the *P. aeruginosa* was mostly isolated from the B and C age groups i.e. elder sheep (table 1).

P. aeruginosa is well known in its resistance against a wide spectrum of antibiotics. In this study, the antibiotic susceptibility of the isolated *P. aeruginosa* strains was tested toward 16 different antibiotics which were comprising recent as well as antibiotics of old generations (Table 2). In the UK, about 18.5% of the *P. aeruginosa* were resistant, or had reduced susceptibility, to some recent antibiotics (3). Although the new antibiotics used in this study, namely: amikacin, ciprofloxacin and the norfloxacin were effective in about 82%, 77% and 71%, respectively and a remarkable number of the susceptible isolates showed, however, an intermediate susceptibility. These results indicate the diminishing of efficacy of these antibiotics against the *P. aeruginosa*. Such findings, indeed, ring the bell about the merging and the developing of some resistance against these new antibiotics, especially that the antibiotic misuse is problematic to the health authorities/enterprises in the world including this country. Synergistic combination of two or more antibiotics is commonly, *in-vitro*, investigated (10). The ciprofloxacin combined with the imipenem did not result to improving the effect of the ciprofloxacin on the *Pseudomonas sp.* while the addition of a third rifampicin antibiotic, results to remarkable synergistic effect (10). In this study, some isolates displayed a high (< 90%) resistance against the majority of the old antibiotics. However, the rest 10% of isolates, which were recorded as susceptible ones, were, indeed, having an intermediate susceptibility. Such findings were obviously, recorded toward the tetracycline, amoxicillin, erythromycin and ampicillin (Table 2).

The bactericidal effect of the serum is mainly referred to the complement effect. In this study, serum resistance of the isolated *P. aeruginosa* was determined against sera of two animal species, namely: the sheep from which the *P. aeruginosa* was isolated and the calf as a foreign species. Although the 14 serum resistant strains showed the same resistance patterns toward the sera of both sources, their count in the calf serum was determined, relatively, with higher logs (2.5-3.5 logs) than that in the sheep serum (2.5 - 2.9 logs). On the other side, the viable count of each of the 3 (18%) serum sensitive isolates was decreased and was determined in lower 1 - 1.8 logs when they were exposed to the untreated calf serum.

This is the first study deals with sheep dermatitis/fleece rot problem in Jordan. The obtained results indicate the importance of further investigation to explore the different circumstances affecting such sheep infection especially that sheep migration between Jordan and the neighbouring countries is not uncommon and does undergone deficient control / quarantine measures.

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