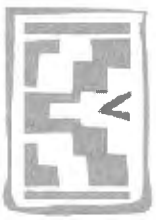

Correlation between ability of *Ornithobacterium rhinotracheale* to agglutinate red blood cells and susceptibility to fosfomycin

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ABSTRACT

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Twenty five freeze-dried isolates of *Ornithobacterium rhinotracheale* were used for the determination of minimum inhibitory concentrations (MIC) against the antibiotic fosfomycin (Fosbac, produced by Bedson SA, consisting of a 25% mixture of fosfomycin). The same isolates were tested for their ability to haemagglutinate chicken red blood cells.

Ten of the 25 isolates were found to be susceptible to fosfomycin (MIC values below 128 ug/mℓ). All of these isolates were able to agglutinate red blood cells. This is the first report on the ability of *O. rhinotracheale* to agglutinate red blood cells.

The remaining 15 isolates were resistant to fosfomycin (MIC values above 128 ug/mℓ). Only five of these isolates were found to have the ability to agglutinate red blood cells.

There appears to be a correlation between the ability of *O. rhinotracheale* isolates to agglutinate red blood cells and their susceptibility to fosfomycin.

The ability of certain isolates of *O. rhinotracheale* to agglutinate red blood cells, raises the questions of differences in virulence between the isolates which can agglutinate red blood cells and those which cannot and the use of this ability to agglutinate red blood cells as an alternative method for serotyping *O. rhinotracheale.*

Keywords: Fosfomycin, *Ornithobacterium rhinotracheale,* red blood cells

INTRODUCTION

Ornithobacterium rhinotracheale was first isolated in 1991 from broilers in South Africa which showed mild respiratory problems and reduced growth rates (Van Beek, Van Empel, Van den Bosch, Storm, Bongers, & Du Preez 1994). Some of the bacteria isolated from these chickens were identified as a new genus and species which Vandamme, Segers, Vancanneyt, Van Hove, Mutters, Hommez, Dewhirst, Paster, Kersters, Falsen, Devriese, Bisgaard, Hinz & Mannheim (1994) classified as *Ornithobacterium rhinotracheale.*

In 1993, Hafez, Kruse, Emele & Sting reported similar bacteria from turkeys and chickens in Germany. During the same year, Charlton, Channing-Santagio, Bickford, Cardona, Chin, Cooper, Droual Jeffrey, Meteyer, Shivaprasad & Walker (1993) reported on the isolation of gram negative pleomorphic bacteria in the USA from chickens, turkeys, a chukar partridge, pheasant and pigeon. In 1993, *O. rhinotracheale* was

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also reported for the first time from broilers and turkeys in the Netherlands (Van Beek *et al.*1994). Vandamme *et al.* (1994) obtained isolates made from the United Kingdom, France, Belgium, Germany and South Africa. Bock, Freidlin, Manoim, Inbar, Frommer, Vandamme & Wilding (1997) also reported on the isolation of this bacterium from Israel.

Little has been published on the antimicrobial sensitivity of *O. rhinotracheale*. In one study, however, it was concluded that acquired antimicrobial resistance was exceptionally frequent in *O. rhinotracheale* (Devriese, Hommez, Vandamme, Kersters & Haesebrouck 1995). Bragg (1998) reported on antibiogram results obtained on isolates of *O. rhinotracheale* in South Africa made since 1996. It was shown that the antibiogram results appeared to correspond well to successful treatments of field infection (De Rosa, Droual, Chin, Shivaprasad & Walker 1996; Hinz, Blome, & Ryll 1994; Hafez *et al.* 1993; Van Beek *et al.* 1994).

There are a number of reports indicating antigenic diversity among isolates of *O. rhinotracheale*. Bock *et al.* (1997), used a rapid serum plate agglutination (RPA) test to identify three different serovars. Van Empel, Van den Bosch, Loeffen, & Storm (1997) used an agar gel precipitation (AGP) test and ELISA to identify seven serovars which were termed A–G. They found that serovar A was the most common isolate from chickens, while isolates from turkeys were equally distributed across all of the serovars. Furthermore, they showed some cross-reactivity between serovars A, B and E. Odor Salem, Pope, Sample, Primm, Vance & Murphy (1997) confirmed that serovar A was the most prevalent in chickens.

On the molecular level, similarities between serovars A and B were found using a PCR based fingerprinting method (Hafez & Beyer 1997) when using two different primers. They could not demonstrate differences between serovar E and G when one set of primers was used. With a particular set of primers, all the serovars were found to be different.

Travers, Coetzee & Gummow (1996) suggested that there are pathogenicity differences between three South African field isolates of *O. rhinotracheale,* but apart from biochemical identification, they made no attempts to characterize these different isolates antigenically. Pathogenicity differences were based on air sacculitis and arthritis lesion scoring techniques. Van Empel *et al.* (1997) suggested that all of the isolates of *O. rhinotracheale* isolated in South Africa were serovar A.

In this study, we investigated the ability of different isolates to agglutinate red blood cells as a method to differentiate between them as well as the correlation between the ability of the isolates to agglutinate red blood cells to sensitivity to the antibiotic fosfomycin (Fosbac).

MATERIAL AND METHODS

Twenty five isolates were biochemically identified as *O. rhinotracheale* using the method described by Vandamme *et al.*(1994). Carbohydrate fermentation patterns for the different isolates were established by inoculating tubes containing phenol red broth, supplemented with 1% (w/v) galactose, glucose, lactose, mannitol, mannose, sorbitol, sucrose and xylose with reconstituted freeze-dried bacteria. These were then incubated at 37°C and observed daily for 7 d in order to determine whether or not each isolate possessed the ability to utilization of the carbohydrate.

Subsequently, minimal inhibitory concentrations (MIC) were performed using a 25% concentration of fosfomycin (Fosbac). An initial antimicrobial concentration of 256 µg/mℓ of the active ingredient was used. A two fold serial dilution was performed in brain heart infusion broth (BHI) until a concentration of 0,25 µg/mℓ was reached. These tests were performed in sterile 96 well plates, as opposed to test tubes. The wells contained 100 µℓ of the diluted antibiotic and were inoculated with 10 µℓ of an 18 h old culture of the different isolates grown in BHI. The plates were incubated in a carbon dioxide rich environment at 37°C for 24 h. As a control, MICs were performed on 16 NAD independent *Haemophilus paragallinarum* isolates in the same manner.

The ability of the isolates of *H. paragallinarum* to agglutinate glutaraldehyde inactivated chicken red blood cells (RBC) has been reported on previously (Kume, Sawata, Nakase & Matsumoto 1983; Eaves, Rogers & Blackall 1989; Blackall, Eaves & Aus 1990; Bragg, Coetzee & Verschoor 1996). The ability of *O. rhinotracheale* to agglutinate glutaraldehyde inactivated RBC was tested according to the methods described by Bragg *et al.* (1996) without modification.

RESULTS

All the isolates were biochemically identified as *O. rhinotracheale* by carbohydrate fermentation tests.

Ten of the 25 isolates of *O. rhinotracheale* were found to be sensitive to fosfomycin and showed MIC's of less than 128 µg/mℓ (Table 1). The mean MIC for these isolates was found to be 59, 4 µg/mℓ and it was found that they all had the ability to agglutinate glutaraldehyde inactivated RBC. Of the remaining 15 isolates which were not sensitive to fosfomycin, only five had the ability to agglutinate RBC.

The 16 NAD independent *Haemophilus paragallinarum* isolates all showed MIC values against fosfomycin between 8–2 µg with the mean MIC of approximately 4 µg/mℓ (data not shown). All of these isolates had been previously shown to agglutinate glutaraldehyde inactivated RBC (Bragg, Greyling & Verschoor 1997).

TABLE 1 Isolate identification, MIC results and ability to agglutinate RBC of different isolates of *O. rhinotracheale*

Isolate identification	MIC (µg/mℓ)	Ability to agglutinate RBC
BB4	64	+
558	16	+
582	32	+
1116	8	+
4G	8	+
1019	6	+
1065	32	+
1282	96	+
1291	64	+
1439	64	+
541	128	–
1568	128	+
735	> 128	–
739	> 128	–
1238	> 128	–
1435	> 128	–
1586	> 128	–
1670	> 128	–
1893	> 128	–
2028	> 128	+
2671	> 128	–
3217	> 128	–
BWO 4	> 128	+
BWO 7	> 128	+
BH2	> 128	+

DISCUSSION

A clear differentiation between the NAD independent variants of *H. paragallinarum* and *O. rhinotracheale* could be established from the MIC values against fosfomycin. All of the *H. paragallinarum* isolates were highly susceptible to this antibiotic and showed MIC values of approximately 4 µg/mℓ. On the other hand, many of the *O. rhinotracheale* isolates were totally resistant to fosfomycin and even at levels as high as 128 µg/mℓ, no inhibition could be detected. Those isolates which showed some susceptibility had a mean MIC of approximately 50 µg/mℓ.

Van Empel *et al.* (1997) suggested that all of the isolates of *O. rhinotracheale* made in South Africa belonged to their serovar A group, which indicates that this group is probably uniform. On the other hand, Travers *et al.* (1996) suggested that there were pathogenicity differences between three different South African isolates. From the results obtained in this study, it would appear that there are least two different groups of *O. rhinotracheale* isolates in South Africa. One of these groups has the ability to agglutinate chicken RBC and shows a higher level of susceptibility to fosfomycin, while the other does not have the ability to agglutinate RBC and shows high levels of resistance to fosfomycin.

This raises the question as to the correlation between the AGP and ELISA based serotyping system used by Van Empel *et al.* (1997) and the ability of these isolates to agglutinate RBC. There is a clear correlation between the RPG serotyping scheme for *H. paragallinarum* proposed by Page (1962) and the hemagglutinin scheme proposed by Kume *et al.* (1983) and refined by Eaves *et al.* (1989) and Blackall *et al.* (1990). It has also been shown that the serogroup A and B isolates of *H. paragallinarum* agglutinate RBC without further treatment. On the other hand, the serogroup C isolates require further treatment before they will agglutinate. There appear to be some similarities to the work done on *H. paragallinarum* and this work on *O. rhinotracheale.*

It has also been shown that there is no cross protection between the different serogroups of *H. paragallinarum.* If there are different groups of *O. rhinotracheale* in South Africa, the question of possible pathogenicity differences between the different isolates is raised. Travers *et al.* (1996) suggested that pathogenicity differences between SA isolates of *O. rhinotracheale* exist, but made no attempt to char acterize the different isolates antigenically. The discovery that some of these isolates posses the ability to agglutinate RBC also raises the question of the possible role that the heamggultinin may play in the protective immunity of the different isolates.

CONCLUSION

There appears to be a correlation between the ability of certain isolates of *O. rhinotracheale* to agglutinate RBC and their susceptibility to antibiotics. It is possible that the same antigen involved in the agglutination of RBC may also be involved in the uptake of fosfomycin (Fosbac).

The ability of *O. rhinotracheale* to agglutinate RBC should be investigated further as a possible alternative method of serotyping the isolates. It is also possible that this ability to agglutinate may also differen tiate between isolates which appear to show differences in pathogenicity, although all of the South African isolates have been shown to belong to the same AGP serovar (Van Empel *et al.* 1997).

This work highlights the need for further research on the serological characterization of *O. rhinotracheale* and the relationship between the different serotyping systems and the pathogenicity and protective potential of *O. rhinotracheale.*

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