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Isolation and characterization of group B streptococci from human and bovine sources within and around Nairobi

J. M. MOSABI,* S. M. ARIMI† AND E. K. KANG'ETHE

Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, College of Agriculture and Veterinary Sciences, University of Nairobi, Kabete, Kenya

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SUMMARY

Group B streptococci (GBS) were isolated from bovine bulk milk and from vaginas and throats of antenatal and postnatal women using TKT and rapid GBS media. Sixty-three of 529 (12%) bovine bulk milk samples, 9 of 48 (19%) vaginal and 3 of 48 (6%) throat samples were positive. Both bovine and human beta haemolytic isolates were characterized biochemically and serologically. Pigment production was a characteristic of both human and bovine beta haemolytic isolates. The majority (88%) of human isolates fermented salicin and not lactose and most bovine isolates were either lactose positive/salicin positive (41%) or lactose positive/salicin negative (38%). Human and bovine isolates were 100% and 85% typable respectively. Serotype distribution was similar in the bovine and human populations with serotype la, lc and lll being most common in both. Fermentation of sugars showed major differences between bovine and human isolates but similarity in serotype distribution suggests some genetic relationship.

INTRODUCTION

Group B streptococci are a well known cause of contagious bovine mastitis leading to economic losses in the dairy industry [1, 2] and despite control measures, their presence continues to cause problems in dairy herds [3, 4]. The infected udder is the main source of infection of healthy cows via contaminated milkers' hands, equipment, udder cloths and milking machines. The organism is also a recognized cause of morbidity and mortality in human beings, particularly infants and adults with predisposing conditions such as neoplasia and nutritional, hormonal or metabolic deficiencies [5–9]. It has also been associated with toxic shock-like syndrome in women [10]. The early onset of the disease is seen mostly in newborn children with low birth weights and in those born prematurely. Late onset disease affects infants 2–4 weeks old and may manifest as meningitis or septicemia. The source of infection for the newborn children is usually the urino-genital tract of the infected mother at birth or later in the nursery. Many healthy individuals, men and women, are carriers of GBS in their urino-genital tract, throat and rectum [11].

Infection of the bovine udder with human strains of group B streptococci causing severe mastitis has been demonstrated [12], but the role of animal strains in human infection is not clear. Studies on serological grouping and serotyping, using carbohydrates and protein cell wall antigens, biochemical and growth characteristics, have shown some common and some different characteristics between the two groups [13–16]. These characteristics are useful in the study of the epidemiology of the disease. DNA/DNA

* Current address: Ministry of Agriculture and Livestock Development, Mariakani Veterinary Investigation Laboratory, P.O. Box 204, Mariakani, Kenya.

† Correspondence address: S. M. Arimi, Department of Public Health, Pharmacology and Toxicology, College of Agriculture and Veterinary Sciences, University of Nairobi, P.O. Box 29053, Kabete, Kenya.

hybridization studies have, however, not shown differences between human and bovine isolates to warrant creation of a new species [17].

This study was undertaken to investigate recovery of group B streptococci from bovine dairy milk, and the vagina and throat of women and, to study the characteristic properties of the isolates from human and bovine sources.

MATERIALS AND METHODS

Isolates of animal origin

Isolation from dairy milk. A total of 529 dairy milk samples were collected from Kenya Cooperative Creameries (KCC), which received milk from a large number of dairy societies and individual farmers from within and around Nairobi. Samples were obtained randomly from the milk delivery containers on reception. A sample (20–30 ml) was taken after thorough mixing, transported to the laboratory in ice-cooled boxes and cultured the same day on TKT medium and rapid GBS medium (Oxoid). TKT medium was prepared by adding to modified Edwards medium (Oxoid) 5% (v/v) sheep red blood cells (washed three times in sterile physiological saline and reconstituted to original volume) and staphylococcal beta toxin. Rapid GBS medium was prepared according to the manufacturer's instructions and dispensed into tubes in 4 ml amounts.

Three loopfuls of milk were streaked onto the TKT agar which was incubated aerobically at 37 °C for 24 h. One millilitre was transferred into rapid GBS medium and incubated under an increased CO_2 concentration (CO_2 gas generating kit, Oxoid BR38) in anaerobic jars at 37 °C for 24 h. Colonies from TKT medium showing haemolysis reminiscent of the CAMP reaction [18] and bacteria from the rapid GBS tubes which showed production of orange-red pigment were purified on blood agar, Gram stained and retested for the CAMP and splitting of aesculin on sheep blood-aesculin agar [19].

Isolates of human origin

Isolation from antenatal and postnatal mothers. The women who participated in this study attended the antenatal or postnatal clinic at Pumwani Maternity Hospital, Nairobi. A high vaginal swab and a throat swab were taken from each of 48 women. The swabs were transported to the laboratory in Stuart's Transport medium (Lab M) and used to inoculate TKT agar after which they were placed in rapid GBS medium. Incubation of inoculated media and isolation were done in the same way as for the milk samples.

Other human isolates. In addition to the group B streptococcal isolates made in this study, 22 more from two other institutions were included in the study. Eight vaginal isolates were provided by P Waiyaki, Kenya Medical Research Institute (KEMRI) and 14 isolates from patients attending Kenyatta National Hospital were provided by I. Wamola, Department of Microbiology, College of Health Sciences (CHS), University of Nairobi.

Test for characteristic properties

Serological grouping. The isolates were grouped by a coagglutination method [20] using latex beads coated with streptococcal group specific antiserum (Oxoid, DR587) to confirm them as group B streptococci. They were grown on blood agar overnight at 37 °C and antigen extracted using freeze dried extraction enzyme (Oxoid DR593) diluted in 2 ml sterile distilled water according to the manufacturer's instructions.

CAMP reaction and aesculin hydrolysis. A single colony picked from a 24 h blood agar culture was tested for the CAMP reaction and splitting of aesculin by streaking on blood aesculin agar [19].

Hydrolysis of sodium hippurate. Tubes showing development of deep blue purple colour within 10 min were considered positive [21].

Pigment production. Purified colonies were inoculated, by stabbing, into 4 ml of the rapid GBS medium in a test tube and the inoculated tubes incubated anaerobically (CO_2 gas generating kit, Oxoid BR38) overnight at 37 °C. Media showing orange-red colour were considered positive.

Fermentation of lactose and salicin. Three millilitres each of 1% lactose and 1% salicin (Alpha Chemicals) in Andrade peptone water (Oxoid) and sterilized by membrane filtration (0·45 μm Millipore), were each inoculated with a pure colony of streptococcus and incubated at 37 °C for 48 h.

Serotyping. Six serotyped strains were provided by R. R. Facklam, Centers for Disease Control (CDC), Atlanta, Georgia. These comprised serotype Ia: 090, CDC-615; Ib: H36B, CDC-618; Ic: A909, CDC-700; II: 18RS21, CDC-619; III: D136C, CDC-620; IV: CDC-1240 and non-typable: NT, CDC-1073. Reference antisera against the serotypes Ia, Ib, Ic, II and III, and polyethylene glycol diluent were also pro-

vided. They were maintained at 4 °C until used. Hot hydrochloric acid antigen extracts of the reference strains were checked for identity using a capillary tube precipitation technique [22].

Antisera for serotyping the human and animal group B streptococcal isolates were prepared from rabbits immunized with antigens prepared from the reference serotypes Ia, Ib, Ic, II, III, IV and non-typable strain [23]. The antisera were absorbed to remove cross reactivity as follows: Ia with Ib and Ic cells; Ib with Ic cells; Ic with Ia cells; and II with Ic cells.

A total of 68 isolates comprising all the 34 haemolytic human isolates and 34 haemolytic animal isolated were serotyped using the rabbit prepared antisera. Antigens were extracted using hot hydrochloric acid and serotyping was done using the capillary tube precipitation technique.

RESULTS

A total of 63 group B streptococcal isolates were obtained from the 529 milk samples. TKT medium recovered the organism from 63 (12%) samples of which 37 were beta haemolytic and 26 non-haemolytic. Rapid GBS medium detected organism from 23 of 529 (4%) samples. All the isolates were haemolytic and all were isolated on TKT medium.

Group B streptococci were isolated from the vagina of 9 (19%) of 48 women and throat of 3 (6%) women. Rapid GBS medium detected all 9 positive vaginal carriers compared to 4 (8%) by TKT medium. The 3 throat carriers, which were also vaginal carriers, were detected by both media. All the human isolates were beta haemolytic.

All the 97 isolates were group B streptococci, CAMP positive and aesculin negative and hydrolysed sodium hippurate. Pigment was produced by all the beta-haemolytic human and bovine isolates but not by non-haemolytic bovine isolates.

To make a comparison of salicin and lactose sugar fermentation, and serotype distribution of human and bovine isolates, the 34 haemolytic human isolates, and an equal number of haemolytic bovine isolates were tested (Table 1). The human isolates fermented salicin and lactose in a pattern that differed from that of the bovine isolates (Table 1). Some human and bovine isolates fermented both sugars. Most (88%) of the human isolates fermented salicin and few (9%) fermented both salicin and lactose. Only one isolate fermented lactose and not salicin. In contrast, most (79%) of the bovine isolates fermented lactose while 41% fermented both lactose and salicin.

Table 1. *Comparison of salicin and lactose fermentation by the haemolytic human and bovine group B streptococcal isolates and their serotype distribution*

Tests	Human isolates ($n = 34$)	Bovine isolates ($n = 34$)
Sugar fermentations		
Salicin + lactose −	27 (79%)	7 (21%)
Salicin − lactose +	1 (3%)	13 (38%)
Salicin + lactose +	3 (9%)	14 (41%)
Salicin − lactose −	3 (9%)	0
Serotypes		
Ia	10 (29%)	16 (47%)
Ib	3 (9%)	2 (6%)
Ic	8 (24%)	4 (12%)
II/Ic	2 (6%)	0
II	3 (9%)	2 (6%)
III	5 (15%)	4 (12%)
IV	3 (9%)	1(3%)
Not typable	0	5 (15%)

All the human isolates were typable. Serotype Ia (29%) was most common followed by Ic (24%) and III (15%). A similar serotype distribution was observed among the 29 typable bovine isolates with serotype Ia (47%) being most prevalent followed by Ic (12%) and III (12%). Of the 12 human isolates, serotype Ia was the most common in both the vagina (44%) and throat (67%) (Table 2).

DISCUSSION

There was no single milk sample which was positive by rapid GBS and which was not isolated on TKT medium. The rapid GBS medium did not, therefore, offer any advantage over TKT medium in recovering group B streptococci from milk. There were probably few organisms in the samples to effect visible colour change in the medium and bacterial contaminants in the milk could have interfered with the pigment production because, when the isolates from these samples were tested later, they produced pigment in the rapid GBS medium. Studies by Wibawan and colleagues [16] showed generally weak pigmentation by bovine strains and a strong pigmentation by human strains. The rapid GBS medium has been developed specifically to detect pigmented beta haemolytic group B streptococci in human clinical specimens [24–26]. In this study, rapid GBS medium was superior

Table 2. *Serotype distribution of the human strains of group B streptococci*

	Source of strains			
	Isolated in this study		Kenya Medical Research Institute	Department of Microbiology College of Health Sciences
Serotype	Vagina ($n = 9$)	Throat ($n = 3$)	Vagina ($n = 8$)	Clinical cases ($n = 14$)
Ia	4*	2*	0	4
Ib	1†	0	2	1
Ic	2	1†	0	5
II/Ic	1	0	0	1
II	0	0	0	3
III	1	0	4	0
IV	0	0	2	0

* Serotype Ia was isolated from the throat and vagina of two of the women.
† The same woman, throat and vagina positive.

to the TKT medium in detecting group B streptococci on vaginal swabs. One problem observed with the TKT medium inoculated with bulk milk was darkening of the medium due to splitting of aesculin. This can interfere with reading of the CAMP reaction [27]. Since group B streptococcus is an obligate intramammary pathogen of the bovine udder, the recovery rate of 12% from bulk milk suggests a sizeable proportion of dairy herds with a group B streptococcal problem and supports the fact that this organism is one of the most common isolates from clinical cases of bovine mastitis in Kenyan herds [28, 29].

The 48 women studied were apparently healthy individuals attending the routine antenatal or postnatal clinics. The vaginal group B streptococcal carrier rate of 19% was within the range reported from other parts of the world [30–33]. Previous studies involving prostitutes and non-prostitutes in Nairobi [34] showed a vaginal carrier rate of 31% and 7% respectively. Although there is very little information on group B streptococcal disease in humans in Kenya, the vaginal carrier level, and the human cases which have been reported [8], suggest that the organism is a potentially important pathogen in Kenya. The throat carrier rate (6%) was much lower than vaginal carrier rate and also lower than that reported elsewhere [7]. The possibility of faecal contamination of the vagina is obviously much greater than contamination of the throat. Interestingly, all the throat carriers were also vaginal carriers. Two of these individuals had the same serotype (type Ia) in their throat and vagina suggesting either contamination of the vagina from gastrointestinal tract or possibly contamination of the throat through the oral route.

In recent years, pigment production by beta haemolytic group B streptococci has received attention as a simple and quick screening test for the presence of the organism in human clinical specimens [24–26] and also as a characteristic for differentiating between human and animal strains [13, 16]. In this study, all the beta haemolytic human and bovine isolates produced comparable amounts of pigment making differentiation between them impossible. However, these findings contrast with those of others who reported that few or none of the animal strains produced pigment, therefore making this characteristic useful for differentiating between human and animal strains [13, 33].

It was observed that most bovine isolates fermented lactose and most human isolates salicin. However, the proportion of isolates fermenting both carbohydrates was higher for bovine than for human isolates. A number of bovine isolates differed from human isolates by fermenting salicin and not lactose. Similar results have been reported by Mhalu [35] and Simmons and Koegh [36]. The latter reported that human isolates were unable to ferment lactose whereas most bovine isolates fermented both carbohydrates. In addition some bovine isolates fermented salicin and not lactose, an observation also made by Butter and

de Moor [7] and Finch and Martin [13]. Although fermentation of the carbohydrates divides group B streptococci into human and animal populations [13], the inconsistency in fermentation makes it an unreliable test to differentiate between group B streptococci from humans and cattle.

From the literature, most human group B streptococci are serotypable compared with those of animal origin which are less often typable [13, 15, 37]. This agrees with our findings since all the human isolates and only 85% of the bovine isolates were typable serologically. The distribution of serotypes among the typable isolates was comparable in human and bovine populations with serotype Ia being most common followed by Ic and III for each population. Serotype II/Ic was found among human isolates and not bovine isolates. Such sharing of antigens has been reported [13].

Of the 12 women positive for group B streptococci, half carried serotype Ia in their vaginas or throats while the other serotypes were distributed among the other 6 individuals. The isolates received from KEMRI were isolated originally from the cervix of prostitutes and non-prostitute women [34]. Among these, serotype III was most common. The isolates obtained from the Medical School, CHS, were from pathological material (type not specified) from patients at Kenyatta National Hospital who had given birth to babies with low birth weights (< 1500 grams). The commonest serotype among these isolates was Ic followed by Ia and II.

Studies elsewhere [8, 9, 38] have most often incriminated serotype III as the major cause of neonatal disease, particularly the late onset form, regardless of whether the carrier rates for other serotypes by women was greater or equal to serotype III. In this study, serotype III ranked third overall in frequency among the women. A case study of newborn infants with meningitis and septicemia at Kenyatta National Hospital, Nairobi [8], reported the recovery of group B streptococci serotype III. Although serotype III may not be the most common in women, it could be, like in other places, a common cause of neonatal disease in Kenya.

Humans can become infected following drinking milk or by contact with the infected cows [39, 40]. Since most of the human isolates fermented salicin and not lactose, while most bovine isolates fermented either salicin or both sugars, the group B streptococci could usually be separated into human and bovine strains using carbohydrate fermentation, but not pigment production or serotyping. Molecular analysis [41, 42], which was not done, may add further information to our understanding of the epidemiology of this organism.

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