Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 4 (1): 1-12, 2009
ISSN 1816-4935

Application of Random Amplification of Polymorphic DNA, Antibiogram and Serotyping for Differentiating *Streptococcus agalactiae* Clinical and Environmental Isolates from Kuwait

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Abstract: The aim of this study was to assess the phenotypic and genotypic diversity among human and environmental isolates of *S. agalactiae* from Kuwait. A total of 87 isolates were collected from clinical and environmental samples. Conventional typing methods were performed by stereotyping test (slid agglutination) and antimicrobial susceptibility test (disk diffusion) method. Molecular typing method was performed by RAPD analysis to study genetic variability at the molecular level. Fifty six of the isolates were positive for *S. agalactiae* by PCR and culture method. The bacterial isolates showed 100% sensitivity to the ampicillin and ciprofloxacin antibiotics, but 75% sensitivity to chloramphenicol and 66% sensitivity to the erythromycin antibiotics. Serotype III was predominant 26.7%, followed by serotype V, Ia and VI. Serotypes found among isolates from environment samples included V 60%, III 40%. Twelve genotypic patterns were generated using a single arbitrary RAPD primer, conventional phenotypic typing methods presented less significant discriminatory power comparing to molecular. Serologic analysis data showed to certain extent correlation with molecular data using genetic clustering and similarity indices generated by RAPD-PCR. The detection of DNA polymorphism between isolates within a serotype confirmed earlier reports of the heterogeneous nature of individual GBS serotypes.

Key words: Molecular typing, genotyping, antibiotic, streptococcosis, Kuwait

INTRODUCTION

Streptococcus agalactiae or group B *Streptococcus* (GBS), is an important pathogen in both human and environmental origin; accounts for substantial number of cases of invasive infections in newborn and young infants, pregnant women and non-pregnant adults who typically have underlying medical conditions (Backer and Edwards, 1995; Schuchat and Wenger, 1994). Currently *S. agalactiae* is the leading cause of bacterial sepsis, pneumonia and meningitis in neonates in the United States and Europe, with a mortality rate of 4 to 6% (Schuchat, 1998, 1999; Trijbels-Smeulders *et al*., 2004). The colonization rate of GBS in pregnant women in Kuwait reported high (Al-Sweih *et al*., 2004). In 1996, the US centers for disease control and prevention issued specific recommendations for GBS prophylaxis, which led to a significant decline in the incidence of early-onset human neonatal infections (Schrag *et al*., 2000; Schuchat, 1999).

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Molecular typing by either randomly amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) or ribotyping revealed a variety of profiles, reflecting the substantial genetic diversity among GBS isolates recovered from human and environmental origin. Application of a recently described RAPD-typing scheme for *S. agalactiae* also provided evidence that a specific human hyperinvasive GBS clone appears to have emerged from a bovine ancestors (Bisharat *et al*., 2004). Most subtyping studies have shown that *S. agalactiae* isolates from human and bovine hosts represent largely separate populations, even though identical subtypes have occasionally been isolated from humans and animals (Lin *et al*., 2000; Martinez *et al*., 2000). Recently, molecular approaches aimed at uncovering the genomic diversity between closely related organisms have been introduced for the analysis of GBS. These include restriction enzyme fragment length polymorphism (RFLP) analysis, ribotyping, plus-field gel electrophoresis (PFGE) and multilocus enzyme electrophoresis (Chatellier *et al*., 1996). However, these techniques possess some drawbacks for the field characterization of GBS isolate. RFLP analysis generates complex banding patterns that are difficult to analyze when the comparison of a large number of strains is intended (Quentin *et al*., 1995). Ribotyping was shown to posses a lower discriminatory power than RFLP analysis or PFGE and multilocus enzyme electrophoresis and labor-intensive methodologies, which restrict their use to reference laboratories (Maslow *et al*., 1993).

Over the last decade, a number of reports on the antimicrobial susceptibility of GBS from veterinary source have been published (Brown and Robert, 1991; Gurein-Faublee *et al*., 2002; Schwarz *et al*., 1994). Resistant of *Streptococcus agalactiae* to erythromycin has increased during the recent years and its increased percent from 19% in 1994 to 46% in 1997 was reported by Hsueh *et al*. (2001).

In this study, the use of serotyping, antibiogram for the susceptibility of antibiotics and RAPD for the genomic analysis of collection of GBS clinical and environmental isolates were evaluated. This RAPD amplification methodology could allow the uncovering of the extent of genetic heterogeneity among clinical and environmental isolates of GBS in our area. PCR protocols were recently developed using randomly intra- and inter-specific differences in bacteria using RAPD (Williams *et al*., 1990).

MATERIALS AND METHODS

Bacterial Isolates

From a total of 56 isolates, 41 clinical, 3 sewage and 12 from different fish tissues (the environmental isolates were cultured from the moribund fishes and sewage water during an outbreak that occurred in Kuwait Bay in August, 2001 and kept frozen at -80°C) provided by Biotechnology Department and Aquaculture, Fisheries and Marine Environmental Department (AFMFD) at the Kuwait Institute for Scientific Research (KISR), the clinical isolates were provided by Al-Adan Hospital (from the collections between 2004 to 2006).

Bacterial Culture

Cultures were maintained on BHIA. When required, the culture was transformed from BHIA plates to BHI broth followed by appropriate incubation for 18-24 h at 37°C (Gherna *et al*., 1992). All cultures were maintained at 4°C in tube with BHI broth (Oxoid) partially solidified with 0.8% agar. Purity was checked by plating on BHI (Oxoid) agar with 0.5% lactose.

DNA Extraction and PCR Amplification

The bacterial cells were harvested from 5 mL brain-heart infusion broth (10^9 cfu mL^{-1}), resuspended in 100 mL TE; total genomic DNA from the entire *Streptococcus* isolates was purified by using a commercial kit (Promega MD, USA).

One set of oligonucleotide primers capable of detecting organisms of genus *Streptococcus* and species *agalactiae* targeting a specific sequence of the 16S RNA gene (Martinez *et al*., 2001) were obtained from Genosys Biotechnologies, UK. The sequences of primers are namely: F1:5'-GAGTTTGATCATGGCTCAG-3' and 1MODA 5`-ACCAACATGTGTTAATTACTC-3' which expected to give an amplicon of the size 220 bp. Amplification of target DNA was carried out in thermal cyclers (Gene Amp. PCR system 7900, Perkin, Elmer, Cetus, USA). All DNA amplification including the appropriate positive and negative controls were done according to the methods of Telenti *et al*. (1993). The PCR products were resolved by agarose gel electrophoresis and photographed under UV light using standard procedure (Qasem *et al*., 2002).

RAPD-Typing

Following screening, six synthetic 10 mer oligonucleotides (Amarsham Pharmacies Biotech) were used in RAPD. PCR-Primer P-2 was selected on the basis of its ability to differentiate GBS genotypes and correlation with specific serotypes.

Amplification reactions were performed in 25 μL reaction mixture using Gene Amp. PCR system 7900, Perkin, Elmer, Cetus. The amplification was performed using a single primer of arbitrary nucleotide sequence P-2 5'- GTTTCGCTCC-3' from Amersham Pharmacia Biotech. The thermal cycler was programmed with a denaturing precycle of 5 min at 95°C, followed by 45 cycles of 1 min at 95°C, 1 min at 36°C and 2 min at 72°C. The RAPD products were resolved by agarose gel electrophoresis and photographed under UV light (Qasem *et al*., 2002).

Gel Pattern Analysis

Photographs of each gel were digitalized with video camera connected to a microcomputer (Alpha ease; Alpha Innotech Corp., San Leandro, Calif, USA). After conversion, the data were normalized and analyzed. Degrees of homology were determined by dice comparisons (Dice coefficient) and clustering correlation coefficients were calculated by unweighted pair group method (UPGMA) Unweighted Pair Group Method with Arithmetic Means). When the calculations were completed, a dendogram showing the hierarchic representation of linkage level between isolates was drawn. The whole process was carried out with molecular analysis software Alpha ease; stant alone version, USA.

Antibiotic Testing

All strains were tested for antibiotic susceptibility to 6 antimicrobial drugs disk diffusion on Muller-Hilton Agar with disks containing (μg/disk) ampicillin (25 μg), ciproflaxin (15 μg), chloramphenicol (30 μg), erythromycin (15 μg), oxytetracycline (30 μg) and rifampicin (30 μg). Antibiotic susceptibility testing was carried out by Bauer *et al*. (1966) method. Strains were recorded as sensitive intermediate resistant according to the interpretation criteria recommended by the National Committee for Clinical Laboratory Standards (CLSI, 2006).

Serogrouping and Serotyping

All the isolates were inspected for β-hemolytic colonies morphologically typical of GBS. Colonies with phenotypic characteristics of GBS were serogrouped using a commercially available latex agglutination kit (Streptex, Remel, Dartford, Kent, UK).

The isolates were serotyped as recommended by the manufacturer, representing each of the five serotypes. All the isolates were serotyped on the basis of capsular polysaccharides by the coagulating method (Kirkegaard and Field, 1997). Anti-type Ia, Ib, II, III, IV and V sera were purchased from Oxoid (Basingstoke, England).

RESULTS

In the present study, a large collection of clinical and environmental isolates belonging to the *S. agalactiae* was diagnosed. Fifty-six isolates were recovered and tested. Eighty percent positive and 20% negative for clinical and 93.3% positive and 6.6% negative for the environmental isolates were reported for *Streptococcus* by culture. Isolates identified as *S. agalactiae* by direct PCR for both clinical and environmental samples showed 100% positive results (Table 1).

Altogether, 6 different antibiotic resistance patterns were observed. A single antibiotic profile was unique, but the other 3 profiles were common to two or more isolates. It is worth noting that clinical and environmental isolates did not share common patterns for erythromycin and oxytetracycline. Resistant to tetracycline was more common among the clinical isolates (9.7%), then environmental (00%), the erythromycin resistant was more in the environmental samples (40%) then the clinical samples (31.7%). All the clinical and environmental isolates showed sensitivity for both ampicillin and ciproflaxin (Table 2, 3).

A highly uneven distribution of clinical and environmental isolates was observed among the 5 clusters. In fact, the largest RAPD type (C) comprised severed clinical isolates (n = 25) and most of the environmental (n = 14) isolates. One RAPD type (E) was heterogeneous and contained isolates of both clinical and environmental origin and as many as 4 RAPD types (A, B and D) were homogenous, containing only isolates of clinical origin.

PCR Identification

The primers 1MOD and F1 specifically amplified a 220 bp DNA segment from the genomic DNA (16S rRNA) of all the reference strains and clinical as well as environmental isolates of *Streptococcus*, but not from other pathogens such as *E. coli*, *Staphylococcus* (data not shown) (Table 1). Forty-one clinical and 15 environmental samples were tested by direct PCR and culture method, in which PCR gave all 56 positive, while in culture only 33 were positive from clinical and 14 out of 15 showed positive from environmental samples.

Antibiogram

All the samples were tested for susceptibility for 6 antibiotics (ampicillin, ciproflaxin, chloramphenicol, erythromycin, oxytetracycline and rifampicin) by using direct PCR method. All the clinical and environmental samples showed 100% susceptibility for ampicillin and ciproflaxin. No resistant to oxytetracycline and rifampicin was found between the environmental isolates, on the other hand the clinical isolates had 9.7 and 2.4% resistance to oxytetracycline and rifampicin, respectively. High resistance was found between clinical samples for chloramphenicol and erythromycin (24.3 and 31.7%, respectively) and 33.3 and 40% in environmental samples (Table 1, 3).

Serotyping

Isolates at 17.8% were nontypeable and the remaining clinical isolates were found to belong to seven different serotypes. Serotype III was the most prevalent representing 26.47% of all isolates. All seven serotypes tested were identical among the 41 clinical isolates (Table 4) and no nontypeable environmental isolates were found. In clinical and environmental serotypes, III and V were the most frequently identified serotypes with prevalence of 26.7 and 25%, respectively. The environmental serotypes shown more homogeneity between isolates, in which nine were from serotype 2 and six from serotype III, nonetheless it was observed that there was high heterogeneity within the serotype using RAPD analysis, serotype V showing two RAPD types F and L and the serotype III showing D, L and C. All the clinical isolates showed varying RAPD types with in the same serotype.

Table 1: Traditional and molecular methods for identification and typing of *S. agalactiae*. Culture and PCR identification of clinical and environmental isolates and the comparison of serotyping, antibiotic-typing and RAPD-typing results for all the *Streptococcus agalactiae* isolates

Source					Antibiogram							
ID	Place	Culture	PCR	RAPD type	Amp	Cip	Cp	Ery	Oxt	Rif	Type	Sero type
Clinical												
454	LR	+	+	I	S	S	S	R	S	S	1	V
475	LR	+	+	D	S	S	S	R	S	S	1	V
478	2024	+	+	D	S	S	R	R	S	S	2	III
398	OPD	-	+	D	S	S	S	S	S	S	6	V
1015	OPD	+	+	H	S	S	S	S	S	S	6	Ib
447	1847	-	+	H	S	S	S	S	S	S	6	Ia
340	1560	+	+	D	S	S	R	S	S	R	5	NT
326	SAB	+	+	C	S	S	S	R	S	S	1	V
1141	W4	+	+	H	S	S	S	S	S	S	6	Ia
1198	SAB	-	+	H	S	S	S	R	S	S	1	Ia
486	LR	+	+	H	S	S	S	S	S	S	6	Ia
1157	4326	+	+	K	S	S	S	S	S	S	6	NT
197	LR	+	+	C	S	S	S	R	R	S	3	III
375	LR	+	+	C	S	S	S	S	S	S	6	III
461	W8	+	+	C	S	S	S	R	R	S	3	III
317	LR	+	+	D	S	S	R	S	S	S	4	NT
452	LR	-	+	B	S	S	R	R	S	S	2	NT
465	LR	-	+	A	S	S	S	S	S	S	6	II
482	SMB	+	+	E	S	S	S	R	R	S	3	NT
467	LR	-	+	B	S	S	R	R	S	S	2	VII
41014	LR	+	+	D	S	S	S	S	S	S	6	III
580	2218	+	+	J	S	S	S	S	S	S	6	III
451	OPD	+	+	B	S	S	S	S	S	S	6	VII
1117	4168	+	+	G	S	S	S	S	S	S	6	II
218	87	+	+	J	S	S	S	R	R	S	3	V
1305	W3	-	+	F	S	S	R	S	S	S	4	II
1054	W8	-	+	G	S	S	R	S	S	S	4	III
557	LR	+	+	H	S	S	S	S	S	S	6	Ia
369	LR	+	+	C	S	S	S	S	S	S	6	III
965	LR	+	+	I	S	S	S	S	S	S	6	VI
379	CR	+	+	I	S	S	S	S	S	S	6	VI
468	CR	+	+	K	S	S	R	S	S	S	4	NT
522	W8	+	+	J	S	S	S	S	S	S	6	NT
314	OPD	+	+	D	S	S	S	S	S	S	6	III
1006	3873	+	+	K	S	S	R	S	S	S	4	IV
323	W8	+	+	E	S	S	S	S	S	S	6	NT
231	OPD	+	+	J	S	S	S	R	S	S	1	NT
522	W8	+	+	G	S	S	S	S	S	S	6	Ia
549	2267	+	+	K	S	S	S	S	S	S	6	VI
216	LR	+	+	A	S	S	R	S	S	S	4	II
230	SAB	+	+	J	S	S	S	R	S	S	1	NT
Environmental												
57	Newaiby	-	+	F	S	S	S	S	S	S	6	V
06	Mullet	+	+	L	S	S	S	R	S	S	1	V
N6Kid	Zobaidy	+	+	F	S	S	R	R	S	S	2	V
60	Mullet	+	+	L	S	S	R	R	S	S	2	V
A1 Wound	Zobaidy	+	+	L	S	S	S	S	S	S	6	V
Ni Kid	Zobaidy	+	+	F	S	S	R	R	S	S	2	V
N6B3	Zobaidy	+	+	F	S	S	R	S	S	S	4	V
66	Zobaidy	+	+	F	S	S	S	S	S	S	6	III
64	Zobaidy	+	+	D	S	S	S	S	S	S	6	III
B2	Mullet	+	+	D	S	S	S	S	S	S	6	III
FK57	Mullet	+	+	L	S	S	R	R	S	S	2	V
65	Mullet	+	+	L	S	S	S	S	S	S	6	V
SW-B3	KB	+	+	L	S	S	S	R	S	S	1	III
SW-14	KB	+	+	L	S	S	S	S	S	S	6	III
SW-B15	KB	+	+	C	S	S	S	S	S	S	6	III

Amp: Ampicillin, Cip: Ciproflaxin, Cp: Chloramphenicol, Ery: Erythromycin, Oxt: Oxytetracycline, Rif: Rifampicin, S: Susceptible, R: Resistance, KB: Kuwait Bay, +: Present, -: Absent

Table 2: Resistance of isolate strains of *Streptococcus agalactiae* to six antibiotics of commercial use, evaluated by disk diffusion method

Antimicrobial agent (μg)	Clinical samples n = 41 (%)	Environmental samples n = 15 (%)
Ampicillin (25 μg)	00 (00)	00 (00)
Ciproflaxin (μg)	00 (00)	00 (00)
Chloramphenicol (30 μg)	10 (24.3)	05 (33.3)
Erythromycin (15 μg)	13 (31.7)	06 (40.0)
Oxytetracycline (30 μg)	04 (09.7)	00 (00)
Rifampicin (30 μg)	01 (02.4)	00 (00)

Table 3: Resistance spectrum to six antibiotics of commercial use taken place by *Streptococcus agalactiae* isolates from clinical and environmental sources

Antibiotic type of resistance	Antibiotic spectrum	Clinical isolates	Environmental isolates
1	Ery	6	2
2	Cp, Ery	3	4
3	Ery, Oxt	4	0
4	Cp	6	1
5	Ery, Rif	1	0
6	0-resistant	21	8

Cp: Chloramphenicol, Ery: Erythromycin, Oxt: Oxytetracycline

Table 4: Percentage variations among the serotypes and antibiogram types for the isolates from clinical and environmental samples

	Antibiogram types						
Serotype	1	2	3	4	5	6	Total % of serotypes
Ia	1	-	-	-	-	5	6 (10.7%)
Ib	-	-	-	-	-	1	1 (1.78%)
II	-	-	-	2	-	2	4(7.14%)
III	1	1	2	1	-	10	15 (26.7%)
IV	-	-	-	1	-	-	1 (1.78%)
V	4	4	1	1	-	4	14 (25%)
VI	-	-	-	-	-	3	3 (5.3%)
VII	-	1	-	-	-	1	2 (3.5%)
NT	2	1	1	2	1	3	10 (17.8%)
Total % of antibiogram	8 (14.2%)	7 (12.5%)	4 (7.1%)	7 (12.5%)	1 (1.78%)	29 (51.7%)	

NT: Non Typeable

RAPD-PCR analysis of 56 unrelated *S. agalactiae* isolates with primers P-2 revealed reproducible polymorphism. Profiles within serotype were clearly distinguishable from patterns observed within other serotypes. Table 1 shows the serotypes specificity of DNA polymorphism generated by primer P-2 electrophoretic patterns generated by primer P-2 revealing sero-specific clustering. A dendogram was generated to illustrate genotypic correlations (Fig. 1) and the genetic relationship among RAPD patterns of *S. agalactiae*. Based on the P-2, primer was represented in the dendogram (Fig. 1). Overall, *S. agalactiae* isolates had 60% similarity. A total of 69.6% of the isolates were clustered in (A to J) with 60-70% similarity among them. One group RAPD-L included with environmental and sewage samples showed 100% similarity. The other RAPD-F included the environmental isolates with 60-70% similarity with clinical isolates. The percent similarity of each group oscillated between 70 and 77%. The heterogeneity of the population showed significant increase by same nongrouped isolates of 40, 18, 19 and 36. General clustering was not observed among the environmental isolates originating from the same source (dead fish or sewage water) except for those from dead fish samples (Zobaidy) 44, 47, 48, 49 and 42 (Fig. 1). Inspite of the existing clinical diversity, it was possible to find three pairs of isolates sharing the same RAPD patterns at 100% similarity in the clinical samples of 4 and 2, 47 and 48, 39 and 35 (Fig. 1).

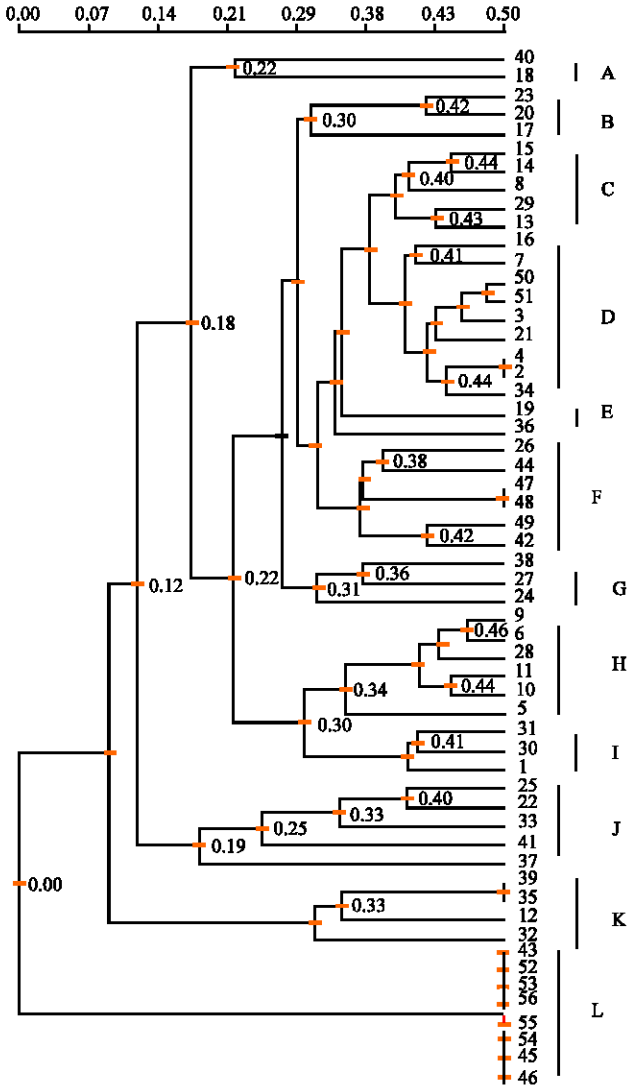

Fig. 1: Dendrogram resulting from a computer-assisted analysis of the RAPD-PCR profiles of *S. agalactiae* isolates recovered from clinical and environmental sources using Primer P-2. The Dice coefficient and a tolerance of 1.00% were used for calculating the similarities and clustering among the profiles using UPGMA method

DISCUSSION

Limited information was available on the molecular epidemiology of Kuwait *S. agalactiae* isolates recovered from clinical and environmental samples (Al-Sweih *et al*., 2004; Duremdez *et al*., 2004). Few studies using DNA-based techniques have been carried out with a large collection of environmental and clinical isolates from Arabian Gulf region. Earlier studies on *S. agalactiae* isolates of human origin and environment origin suggested that RAPD is superior to serotyping for epidemiological evolution of this pathogen (Chatellier *et al*., 1997; Limansky *et al*., 1998).

In the present study, RAPD was used to study a large collection of human (clinical) and environmental isolates from Kuwait. In general, high genetic diversity was found between the clinical isolates. A possible explanation for this diversity is that different isolates originated from different source of patient (Al-Sweih *et al*., 2005). *S. agalactiae* can be differentiated on the basis of different polysaccharide, surface antigens. In this study and earlier study (Al-Sweih *et al*., 2005) serotype III was the most important, earlier studies showed a relative heterogeneity in the distribution of different serotype of clinical isolates (Kirkegaard and Field, 1977). The importance of invasive serotype III strains is well known among human isolates (Quentin *et al*., 1995; Selin *et al*., 1992) but the significance from environment isolates is yet unknown.

In general, no evidence of correlation could be established between serotyping and RAPD patterns. This is the first study available for clinical and environmental isolates which combined genomic diversity and antigenic typing for isolates from Kuwait. Results showed genetic heterogeneity, not only among different serotypes, but also among isolates belonging to same serotype. This suggests that the RAPD technique may be more accurate than capsular serotyping in differentiating *S. agalactiae* of clinical and environmental origin; therefore it appears to be of great value for epidemiological studies.

In the present study, at least 4 isolates from clinical samples had identical RAPD patterns i.e., 2, 4 with serotype I and IV and 38, 39 with serotypes IV and VI, respectively. Five environmental isolates, 42, 44, 47, 48, 49 with 47 and 48 identical RAPD pattern formed a cluster-F with similarity to cluster-D. All the environmental isolates except for these mentioned, formed RAPD cluster-L with 100% similarity. There may be a common source of *S. agalactiae* in different samples from the same source (Duremdez *et al*., 2004).

The serotype distribution of *S. agalactiae* of human origin appeared to have changed one time. Until recently, the predominant serotypes that were detected among clinical isolates were Ia and III (Al-Sweih *et al*., 2005; Selin *et al*., 1992). A striking change however occurred in the 1990s, when the percentage of serotype V reported to be from clinical, 2.6% in 1992 to 14% in 1993 and then to 20% in 1994 (Harrison *et al*., 1995).

The reason for this increase is still unclear; interestingly serotypes V and III were identified in the present study and proved to be the most frequent serotypes among isolates with prevalence of 26.7 and 29%, respectively.

In this study, only one pair of environmental, 47 and 48 (serotypes IV and II) had 100% RAPD pattern similarity. Only one clinical isolate, 13 (serotype I) was clustered with RAPD-F type. Also the isolates, 54, 55 and 56, from sewage water samples were clustered in RAPD-L type with isolates of fish samples. This is in agreement with the results of Jenson and Aarestrup (1996) who detected identical ribotypes for isolates from milk and dairy workers. This suggests the possibility of common origin for both isolates. RAPD cluster-L showed isolates belonging to different serotypes, but indistinguishable by genetic analysis. This was described by Bingen *et al*. (1992) and Jensen and Aarestrup (1996) one possible explanation is the ability of *S. agalactiae* to regulate capsule expression in phase shift-like manner (Selin *et al*., 1992).

In summary, the collection of human and environmental GBS included in the present study was heterogeneous with regard to phenotypic characteristics with the antimicrobial resistance. In the present study, resistance to erythromycin in clinical and environmental isolates were 31.7 and 40%, respectively and chloramphenicol 24 and 33.3%, respectively. This was observed in considerable proportion among GBS isolates from human (clinical) and environmental samples. Ampicillin and ciproflaxin have been favorably selected in the treatment of both human and bovine *S. agalactiae* infections; however, for ampicillin and ciproflaxin-allergic individuals, erythromycin and chloramphenicol are recommended. The prevalence of resistance to erythromycin and chloramphenicol has been increasing in *S. agalactiae*. On the other hand, the homogeneous susceptibility of ampicillin

and ciproflaxin corroborates the concept that β-lactams still constitute good prophylactic and therapeutic options, since resistance to these antimicrobial agents has not emerged in *S. agalactiae* strains. While it is almost undisputed that extensive use of antibiotics in medicine and animal husbandry results in increased antibiotics resistance among bacterial populations, evidence that antibiotic usage in farm animals contributes to the emergence of human antibiotic-resistant pathogen is more limited, several studies have suggested that antimicrobial use with animals influences development of antibiotic resistance among pathogens in humans (Aarestrup, 1999; Witte, 1998).

The significant prevalence of GBS isolates resistant to erythromycin and chloramphenicol indicates the limitation of therapeutic use of these antimicrobials for the treatment and suggests that measures of control and prevention, including the control of the use of antimicrobials, may not have been effectively applied. Data on antimicrobial susceptibility are valuable for monitoring the emergence of resistance traits and for guiding the selection of more judicious and effective therapy. In the present study, resistance to erythromycin and chloramphenicol was observed in considerable proportion in both clinical and environmental samples, respectively. The high rate of tetracycline resistance (100%) that was observed among the erythromycin resistant *S. agalactiae* isolates was also noted in some earlier reports (>80% in Canada, 89.1% in France, 87% in Spain and 99.2% in Taiwan). Nonetheless, the mechanisms of this coexistence are yet unclear (Culebras *et al*., 2002). On the other hand, the chloramphenicol resistance rate that was detected in this study between the clinical isolates (24.3%) was considerably higher than those previously reported from Taiwan, 20.3% (Wu *et al*., 1997) and the United States (1%) (Murodoch and Reller, 2001). This rate was also considerably higher than that detected in Turkish *S. pyrogenes* isolates (5.6%), in which tetracycline resistance was 39% (Acikgoz *et al*., 2003). However, to assess the statistical significance of erythromycin-chloramphenicol co-resistance detected in these isolates, the erythromycin-susceptible isolates should be compared with the resistant ones.

Observations reinforced the importance of using more than a single molecular technique as the basis for assessment of the genetic relationship among isolates, given that isolates that are concordantly grouped into similar types by different systems are increasingly more likely to be highly related. This was of particular significance in relation to interpretation of RAPD-PCR profiling, since criteria for the interpretation of results obtained on the use of this technique have not been proposed. Analysis of both RAPD-PCR and antibiogram profiles and of the resulting dendograms showed that isolates obtained from the same source presented identical or highly related DNA profiles and composed the same groups of similarity, with only few exceptions.

Results confirmed the earlier studies that PCR is more sensitive then culture and suitable for detection of *S. agalactiae* in environmental and clinical specimens (Duremdez *et al*., 2004). Therefore, results would indicate that the PCR-based technique may be used as a reliable alternative method to conventional culture method for species identification of GBS from human and environmental origin. Different RAPD-PCR approaches with variety of primers have been used for typing GBS recovered from bovine and human sources (Culebras *et al*., 2002).

Genotypic techniques examined DNA differences between microorganisms of the same family and were considered superior to phenotypic techniques in that they can more accurately identify epidemiologically related strains (Lipuma, 1998). Further, phenotypic techniques often lack the typeability, reproducibility and discriminatory power that genotypic methods have (Maslow *et al*., 1993). For example, because many GBS strains within a serotype can be genetically similar (Quentin *et al*., 1995) and most disease-causing microorganisms are relatively homogeneous when compared to other lineages (Musser, 1996), genotypic techniques are extremely helpful to identify subtle genetic differences between strains.

Genotype, serotype and antibiogram correlations remain useful, as certain serotypes have been closely correlated with clinical significance (Wessels and Kasper, 1993). However, serotyping is relatively expensive and often difficult procedure to perform. As an alternative typing strategy, RAPD-PCR assay is relatively simple to perform and has the advantage of being able to differentiate strains within specific serotypes.

This study was able to demonstrate the importance of RAPD analysis in the investigation of its discriminatory power and the genetic polymorphism of the pathogenic isolates belonging to the species *S. agalactiae* from environment and clinical source.

Of date, this is the first report to describe phenotypic and genotypic characteristics, as well as the antimicrobial resistance and the respective genetic determinants, of *S. agalactiae* isolates from human and environmental origin in Kuwait. Detailed genetic analysis of GBS strains isolated using similar molecular techniques in other areas of the country and around the world may contribute to a better understanding of the biological diversity and epidemiological aspects involved in the transmission of GBS diseases from a regional and global perspective.

ACKNOWLEDGMENTS

Thanks are extended to Al-Adan hospital and the Kuwait Institute for Scientific Research for providing us with the samples. We would like also to express of sincere thanks to Mr. Mohamed I. Qasem for his help in technical writing of the manuscript.

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