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ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF *STREPTOCOCCUS AGALACTIAE* ISOLATED FROM DIFFERENT CLINICAL SPECIMENS

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Abstract

Streptococcus agalactiae, commonly known as Group B Streptococcus (GBS) is a major etiological agent of invasive neonatal infections worldwide and poses a significant threat to pregnant women and immunocompromised adults. Screening in pregnancy helps determine the need for prophylaxis to prevent neonatal transmission. The present study was conducted to study the prevalence and antimicrobial resistance patterns in GBS isolated from various clinical samples collected from Hyderabad, Sindh. Bacterial isolation and identification were done by using cultural, microscopic, and biochemical testing. The serogrouping was performed using PathoDxtra Strep Grouping Kit (Thermo Scientific). Antibiotic sensitivity profiling was carried out using Kirby-Baur Disc diffusion method. Identification using culturing and immunological testing and sero-grouping revealed seven different groups of Streptococci based on Lancefield grouping system. Among them a total of 117 isolates were GBS which were recovered from urine, blood, pus, HVS, and Swabs samples. Gender wise distribution of patients from whom the GBS positive samples were collected showed that 46.15% (n=54) samples were from male and 53.85% (n=63) belonged to female patients while all age groups were sensitive to GBS infections with higher frequency in 0-10 years age group. All isolates of GBS were highly sensitive to vancomycin (100%), followed by penicillin and ceftriaxone (99.14%). However, resistance against clindamycin, and erythromycin, was observed with varying patterns of sensitivity against them. Overall data suggested that although the front-line antibiotics are still largely effective for GBS infections, the sporadic cases of antibiotic resistant GBS and the increasing resistance against lincosamides and macrolides could be challenging for public health. Therefore, frequent local surveillance studies are highly recommended.

Key Words: *Antibiotic resistance, Group B Streptococcus (GBS), Streptococcus agalactiae*

INTRODUCTION

The genus Streptococcus comprises Gram-positive cocci shaped bacteria typically arranged in chains. Certain species, such as *Streptococcus agalactiae* may exhibit sporadic zoonotic potential, although they independently cause disease in humans and animals. *S. agalactiae* is classified as group B Streptococcus (GBS) by Lancefield grouping system. It is a leading cause of invasive infections including septicaemia, meningitis, and pneumonia in newborns. Neonatal colonization typically occurs during childbirth, when the infant inhales or swallows amniotic fluid contaminated with GBS (1). GBS usually are asymptomatic colonizer of the digestive and genitourinary tract in healthy adults. However, these bacteria can lead to severe invasive infections particularly in neonates and immunocompromised individuals. The earliest documented cases of GBS as human pathogen appeared in the late 1930s, with reports describing three fatal incidences of puerperal sepsis caused by this bacterium. The lungs serve as a primary entry point for the bacteria into the bloodstream, as GBS is capable of adhering to and invading both alveolar epithelial cells (2) and endothelial cells (3).

Various antibiotic classes are used to treat streptococcal infections including *S. agalactiae* associated infections. Front-line antibiotics include β- lactam antibiotics such as penicillins (alone or with aminoglycosides) and cephalosporins (cefozolin or ceftriazone). Macrolides, lincosamides, fluoroquinolones, and tetracyclines also serving as the front-line options particularly for people having allergies to penicillin. Given the limited role of aminoglycosides such as amikacin as monotherapy for Streptococci, aminoglycoside should therefore be investigated in the context of combination therapy only (4, 5). Antibiotic

pressure which usually leads to the emergence of resistance acquisition remains slower in *S. agalactiae* compared to the members of *Enterobacteriaceae* family, likely due to limited horizontal gene transfer. However, sporadic cases of penicillin-resistant GBS indicates about the tendency of the development of antibiotic resistance in GBS isolates due to mutation (6). Consequently, locality-wise routine screening for antibiotic susceptibility patterns in GBS isolates becomes essential, particularly in Pakistan where data about the antibiotic susceptibility patterns in GBS isolates is limited. Here, this study aimed to determine the isolation frequency of GBS and to understand the current antibiotic susceptibility patterns in *S. agalactiae* isolates from Sindh. The findings will be helpful in empirical therapy and contribute to break the AMR campaign.

MATERIALS AND METHODS

A total of *n*=117 clinical isolates of GBS were isolated from different clinical samples of blood, urine, pus, wound swabs, and high vaginal swab (HVS) collected during one year from different diagnostic laboratories located at Sindh. The samples from patients suspected of bacterial infections by a local physician based on particular signs and symptoms were included. All samples were processed for the isolation of *S. agalactiae* (GBS), using standards microbiological methods (7). The identification was carried out using cultural, biochemical and immunological tests at Clinical and Molecular Microbiology Research Laboratory (CMRL), Institute of Microbiology, University of Sindh, Jamshoro.

IDENTIFICATION OF STREPTOCOCCI

Initial identification of isolates at genus level was carried out based on colonial characteristics on solid media, and microscopic characteristics of the clinical isolates which included determination of Gram's reaction, shape, arrangement (they were single, in pairs, in tetrads, or in chains) (8). Then biochemical characteristics were studied through different tests that included: CAMP test, Bacitracin test, catalase test, bile esculin test, and blood haemolysis test.

SEROGROUPING USING PATHODXTRA GROUPING KIT

Bacterial isolates identified as Streptococci were initially classified based on their haemolytic properties observed on blood Agar. Further identification was accomplished through Lancefield grouping, which is based on the identification of specific carbohydrate antigens present on the bacterial cell wall. PathoDxtra™ Strep Grouping kit (Thermo Fisher Scientific), a rapid latex agglutination assay was used to classify clinically significant streptococcal isolates. Briefly, one test tube for each specimen was used and 1 drop of Reagent 1 was added to each specimen tube. 1 to 4 isolated ß-haemolytic colonies were selected and resuspend in reagent 1. The mixture was thoroughly mixed to ensure turbidity. Next, one drop of Reagent 2 was added to each tube, and the contents were mixed by gently tapping the tube for five to ten seconds. Then, five drops of reagent 3 were added, and the tube was again mixed for additional five to ten seconds. On the PathoDxtra reaction slide, a designated row of test circles was assigned for each specimen. An aliquot of 40-50µl of the prepared extract was dispended into each of six test circles. The latex reagents were gently resuspended by inversion prior to use. One drop of Strep A Latex was added to the first circle, followed by Strep B C, D, F and G Latex regents to the subsequent circles in the same sequence. The latex reagents and extracts in each circle were thoroughly mixed using separate mixing sticks. The slide was then gently rocked back and forth under adequate lightening conditions to facilitate the observation of agglutination. A positive agglutination reactions withing 30 seconds indicated the specific Lancefield group.

ANTIBIOTIC SUSCEPTIBILITY TESTING

Antibiotic susceptibility testing (AST) was carried out using the Kirby-Bauer disk diffusion method as described previously (9), in accordance with CLSI guidelines (10). Briefly, Mueller-Hinton Agar plates were used as a growth medium, and commercially available antibiotic-impregnated filter paper discs containing defined concentration of antibiotics were employed. A bacterial culture suspension was uniformly spread across the surface of MHA plate using a sterile swab to ensure even distribution of the

bacterial culture. Within 15 minutes of inoculation, antibiotic discs were aseptically placed on the agar surface using sterile forceps. The plates were then incubated at 37°C for 24 hours. The next day, zones of growth inhibition around antibiotic disks on bacterial lawn was observed and measured for analysis as per CLSI guidelines.

RESULTS

A total of 117 GBS (*S. agalactiae)* isolates were recovered from different clinical samples including blood, urine, pus, wound swabs, and high vaginal swab (HVS), specimens collected from 18 different areas of Sindh covering most of the Sindh except Karachi. The highest prevalence was recorded in Hyderabad, followed by Jamshoro and Sukkur cities of Sindh. All the samples were distributed month wise to determine the seasonal effects on the prevalence of streptococcal infections. The *S. agalactiae* was isolated from clinical samples collected from both male and female patients. Data showed that 46.15% of GBS were recovered from male patients while 53.85% belonged to female patients. The range of the age of patients with S. *agalactiae* associated infections was 0-80 years with a mean of 31.49 ±19.93 for age value of female patients and 30.42±19.83 for male patients. The age wise distribution of patients with GBS positive result showed that most of the *S. agalactiae* associated infections was occurring in age group of less than ten years of age, followed by the age groups 21-30 and 31-40 years of age. The lowest prevalence was recorded from the age group of 61 and above (Fig. 1). Furthermore, the highest infection rate was observed during April and May and then October to December months of the year. It was observed that a significantly (p- value <0.05) higher frequency of GBS isolates belonged to urine samples as compared to other specimens such as pus and blood. However, least frequency of GBS isolation was observed from HVS and wound swab specimens (Table I).

Table I. Frequency of GBS isolates from various clinical specimens

Clinical specimens	%	n
Blood	17.09	20
Urine	52.99	62
Pus	23.93	28
HVS	4.27	5
Wound Swab	1.71	2
Total	100	117

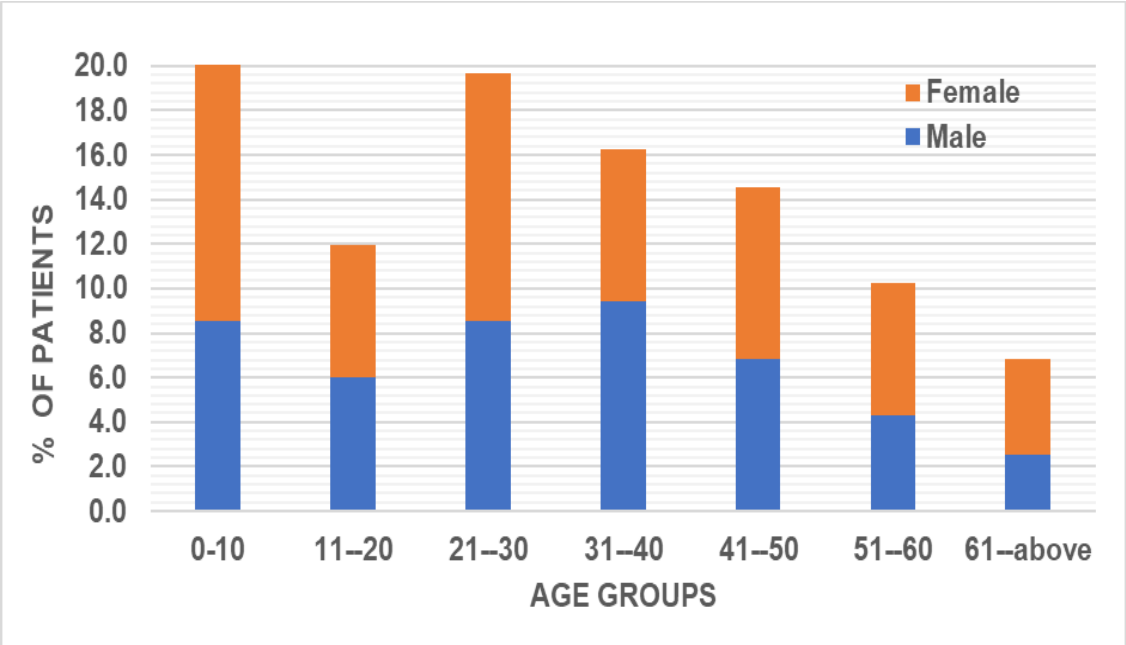

Fig. 1. Age-wise distribution of patients with GBS associated infections

The PathoDxtra™ Strep Grouping kit confirmed that the 117 isolates belonged to GBS. For the identification and characterization of GBS (*S. agalactiae*), the isolates were maintained on blood agar plates (Fig. 2).

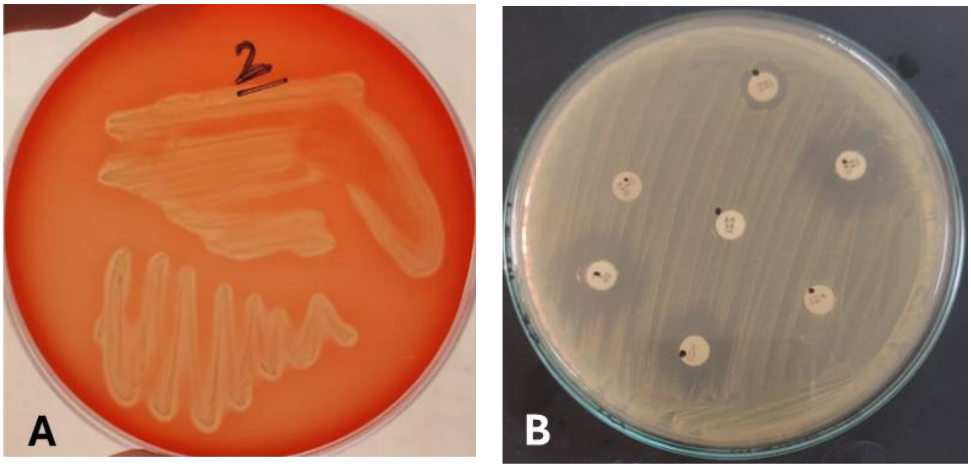

Fig. 2. Representative figures of (a) pure culture of GBS on blood agar and (b) antibiotic susceptibility testing

All the isolated GBS (*S. agalactiae*) were assessed for their antibiotic susceptibility patterns. AST data of the present study demonstrated high sensitivity of *S. agalactiae* isolates against penicillin and vancomycin Table 2. However, increasing trend of resistance was found against clindamycin, and erythromycin, thus varying patterns of sensitivity against them was observed. When Ofloxacin was used as analogy of levofloxacin. 23.% isolates were resistant (Table II). Our findings are supported with the (11), who showed that amikacin and erythromycin resistance has increased significantly in MDR CC17 GBS sub-lineage, which exhibits resistance to tetracyclines, macrolides, lincosamides, and amikacin as a result of the replacement of the pilus island 1 genetic locus by mobile genetic elements carrying the resistance determinants *tet*(O), *erm*(B), and *aphA-3* (11). Moreover, ceftriaxone was also found effective showing .99.14% efficacy against GBS isolates of this study. Overall data suggested that although the front-line antibiotics are still largely effective for GBS infections, the increasing resistance against lincosamides and macrolides is challenging.

Table II. Antibiotic susceptibility patterns of GBS isolates

Antibiotics	Group of antibiotics	Sensitive		Resistant	
		%	(=*n*)	%	(=*n*)
Clindamycin	Lincosamides	84.62	99	15.38	18
Ceftriaxone	Cephalosporins	99.14	116	0.86	1
Erythromycin	Macrolides	81.20	95	18.80	22
Ofloxacin	Fluroquinolones	76.07	89	23.93	28
Penicillin	Penicillins	99.14	116	0.86	1
Vancomycin	Glycopeptides	100.00	117	0.00	0

DISCUSSION

The present study was conducted to determine the isolation frequency of GBS and to understand the current antibiotic susceptibility patterns in *S. agalactiae* (GBS) isolates from various clinical samples from various parts of Sindh. A total of 117 clinical isolates of *S. agalactiae* were recovered and included in the present study, identification based on cultural, biochemical and Lancefield grouping using latex agglutination test, demonstrated prevalence of GBS in various clinical samples. AST data demonstrated that all isolates of GBS were highly sensitive to vancomycin (100%), followed by penicillin, and ceftriaxone. Clindamycin, erythromycin and ofloxacin were also effective, however, varying patterns of sensitivity were recorded against them.

The data of AST patterns of GBS demonstrated that β-lactam group antibiotics showed excellent efficacy with penicillin and cephalosporins 99.14% , a finding that accords with the large body of recent surveillance showing continued predominance of penicillin-susceptible GBS and preserved third-generation cephalosporin activity in most regions (6, 12) . However, a number of current multinational and single-centre studies have emphasized upon the emergence of isolates with reduced penicillin susceptibility (increasing penicillin MICs) in distinct settings, so unusual penicillin results should prompt confirmatory MIC testing and careful reporting of breakpoints used (13, 14).

The observed intermediate-to-lower susceptibilities to macrolides and lincosamides (erythromycin 81.2%, clindamycin 84.6%) reflect the widely reported, geographically heterogeneous rise in macrolide/clindamycin resistance in GBS; this trend has clinical impact because erythromycin and clindamycin are recommended alternatives for intrapartum prophylaxis in penicillin-allergic patients, and inducible MLS_B phenotypes (D-test positive) may lead to clindamycin therapeutic failures if unrecognized (6, 15). Moreover, the reduced susceptibility to fluoroquinolones (ofloxacin 76.1%) is in agreement with various published reports showing increasing fluoroquinolone resistance or reduced susceptibility in GBS isolates over the last decade, and argues against empirical use of fluoroquinolones for maternal or neonatal GBS infections without MIC confirmation (12, 16). In summary, these data support continued use of β-lactams for empirical management where GBS is suspected, and highlight the need to verify penicillin and ceftriaxone MICs when atypical results are observed, compare findings with current local antibiograms to recommend intrapartum prophylaxis policies and stewardship interventions (17).

CONCLUSION

S. agalactiae isolates of this study exhibited high susceptibility to β- lactam and glycopeptide antibiotics particularly penicillin, ceftriaxone and vancomycin, underscoring their continued efficacy as 1st line therapeutic options. On the other hand, increased resistance rates were observed against clindamycin and erythromycin. These findings highlight the dire need for rational antibiotic use, continuous antimicrobial susceptibility surveillance, and implementation of effective stewardship programs to curb the further development and dissemination of resistant in GBS isolates.

Conflict of interest:

The authors declare no conflict of interest.

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Authors' contribution:

SAM carried out laboratory work and data collection; SB wrote the paper and analyzed data and interpretation of results; SAT reviewed the manuscript, and supervised the study, SKB helped in data collection and analysis. All authors read and approved the final version of the manuscript.

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