Original Article

Distribution Of Virulence Factors Genes And Their Association With Antibiotics Resistance In Streptococcus Agalactiae Isolated From Pregnant Women In Al Anbar City

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Abstract

Group B streptococci (GBS) are globally recognized to cause adverse pregnancy outcomes, such as stillbirths and miscarriages, and are one of the main causes of newborn sepsis and meningitis. The high resistance of GBS to antibiotics becomes difficult or impossible to treat, becoming increasingly common, causing a global health crisis. It complicates their eradication, potentially leading to the development of chronic infections. A total of 181 specimens were obtained from pregnant women. Out of these specimens, 22 isolates were bacteriologically identified as S.agalactiae. They were collected from Al-Anbar Province hospitals. Twenty-two isolates were identified as GBS depending on cultural and microscopical properties, automated (VITEK-2 system), and molecular identification based on atr gene, which is an essential gene expressed isolates in all S.agalactiae. Polymerase chain reaction (PCR) was used to detect the prevalence of virulence factors and antibiotic resistance genes; ermB gene was found in 22/22(100%), hly gene in twenty-one isolates (95.45%), scpB also found in twenty-one isolates (95.45%), tet gene (95.45%), mefA gene 12/22(54.54%), Rib gene was found only in five isolates (22.72%), bca found in three isolates only (13.63%), bac not found in any isolates(0%).

Keywords: - Streptococcus agalactiae, Virulence factors, antibiotic resistance genes.

1 INTRODUCTION

Streptococcus agalactiae is an important cause of mortality and morbidity In neonates, maternal women, the elderly, and immunocompromised adults (Saad et al., 2018). (Saad et al., 2018). S.agalactiae causes infections in women during pregnancy and puerperium and invasive infections in newborns (Del Carmen Palacios-Saucedo et al., 2022). Colonization of the mother is the main factor in mother-to-child GBS transmission(Seale et al., 2017). S.agalactiae has a greater ability to cause a various array of infections depending on a multitude of virulence agents acting to evade the host's immune system, promote cell adhesion, maximize dissemination, adapt to alternative environments, and function in several pathogenic pathways (Landwehr-Kenzel & Henneke, 2014). Virulence factors include capsular polysaccharides (CPSs), surface-localized proteins, regulatory proteins, and toxins (Safadi et al., 2011). Many different virulence factors are used by S. agalactiae, such as pore-forming toxins (-hemolysin/cytolysin and CAMP factor) that harm host cells, adhesion factors (FbsA fibrinogen-binding protein, C protein, Rib protein, and HylB hyaluronidase) that promote adherence to cells or the extracellular matrix, and factors (such as C protein) that reduce(Łysakowska et al., 2013), and factors that make it possible to escape the host immunological reaction (ability to produce C5a peptidase, CspA serine protease) CPSs have a significant role as virulence factors and are essential targets for developing vaccination methods (Le Doare & Heath, 2013). Antimicrobial resistance (AMR) is a serious threat to public health worldwide because of its global spread. AMR not only substantially raises the cost of providing medical treatment but also increases mortality and morbidity. The antimicrobial drugs become less effective gradually because of the unnecessary use of antibiotics(Zhu et al., 2022). Macrolides (erythromycin) and lincosamide (clindamycin) were used as a second line of antibiotics that are usually prescribed for those with an allergy to beta-lactams or who have GBS penicillin resistance which was used as the first line to treatment (Hayes et al., 2020). Activated drug efflux controlled by the mef(A) gene or modification of the drug target on the rRNA caused by methylases produced by the erm genes are the two main causes of resistance to macrolides in GBS (Wang et al., 2015).

2 MATERIALS AND METHODS

Sample collection

Samples were collected from August 2021 to the end of December 2021, by collecting 181 samples from pregnant women in the third trimester of pregnancy, the specimens include vaginal swabs from females admitted into Al-Anbar Province hospitals.

Identification of S. agalactiae

A total of 181 isolates from the vaginal swabs were collected. were cultured by strewing them onto a plate of agar containing 5% sheep blood. For 18–24 hours, the plates were incubated at 37 °C in 5% CO2. Using common microbiological S. agalactiae morphological identification procedures, including the Gram stain and the Catalase test, the isolates were recognized as GBS. CAMP test, bacitracin test, automated identification by (VITEK-2 system), and molecular identification based on *atr* gene

DNA isolation and quantification

Genomic DNA was extracted from bacterial culture using DNA isolation kits (Geneaid, Korea) according to the manufacturer's instructions. DNA concentration and purity were determined using a Nano-drop device and stored at 20 °C to prevent degradation. According to manufacturer's instructions, 1 X (TAE) buffer, 1% agarose gel, and molecular weight markers (100 bp) were all prepared.

PCR reactions mixtures and conditions

Amplification of *atr* gene was done using standard PCR and atr primers 5'-CAA CGA TTC TCT CAG CTT TGT TAA-3' and 5'-TAA GAA ATC TCT TGT GCG GAT TTC-3', *bac* primers F- CTA TTT TTG ATA TTG ACA ATG CAA, and R- GTC GTT ACT TCC TTG AGA TGTAAC, *bca* primers F- TAA CAG TTA TGA TAC TTC ACAGAC, and R- ACG ACT TTC TTC CGT CCA CTTAGG, *ermB* primers F- GAA AAG GTA CTC AAC CAA ATA, and R- AGT AAC GGT ACT TAA ATT GTTTAC, *hly* primers F- TCC ATT TAA AGC CCT TGG TG, and R- GGC GCC AGT ATA AGC AAC AT, *mefA* primers F- AGT ATC ATT AAT CAC TAG TGC, and R- TTC TTC TGG TAC TAA AAG TGG, *Rib* primers F- CAG GAA GTG CTG TTA CGT TAA AC, and R- CGT CCC ATT TAG GGT CTT CC, *scpB* primers F- ACA ACG GAA GGC GCT ACT GTT C, and R- ACC TGG TGT TTG ACC TGA ACT A, *tet* primers F- GTG GAG TAC TAC ATT TAC GAG, and R- GAA GCG GAT CAC TAT CTG AG (Mudzana et al., 2021). 20 µl reaction mixture was all done according to the manufacturer's instructions (BIONEER, Korea). Conditions for pCR thermal cycling included a first denaturation phase lasting 4 minutes at 94 °C and 35 cycles {denaturation 94 °C for 1 min, annealing at 58 °C for 45 sec, extension 72 °C for 1 min} and a final elongation step at 72 °C for 7 min.

3 RESULTS AND DISCUSSIONS

Isolation of S. agalactiae

This study has taken four months from the beginning of August and completed with the end of December 2021. From 181 clinical specimens, there are twenty-two isolates were identified as GBS. The isolation rate of GBS from pregnant women was (12.15%), and most of the participants were between the age range of 25 - 37 years. With regard to the clinical history of the participants, the participants had multigravida(54.54%) or abortion(22.73%) or stillbirth(9.09%) or neonatal death(13.64%). The rate of S.agalactiae isolated from pregnant women depends on many factors such as virulence of isolates, health status of patients, impact of environmental factors and hormonal changes that occur during pregnancy, and the resulting microbiota imbalance that raises the risk of GBS infections, which can lead to complications for both mothers and their children. Many local studies were shown the rates of GBS in Iraq, such as Hassan explained the rate of GBS in Baghdad (18%)(Hassan et al., 2019).Also, there are international studies that show GBS rates with explaining the clinical history of the patients. Such as in Southeast Ethiopia, the prevalence of S.agalactiae based on the clinical history of the patients which was (75.8%) were multigravida, (25.3%) had a history of abortion, (12.1%) had a history of stillbirth, and (15.4%) had a history of neonatal death(Tesfaye et al., 2022). The prevalence rate was in Egypt (17.89%) and Kuwait (16.4%) (Abdallah et al., 2021).

Identification of S. agalactiae

The results of tests for identifying S.agalactiae using microscopic diagnostics was positive Coccus (chain or pair) and negative to catalase. S. agalactiae is β-hemolytic on blood agar. Major virulence factor employed by GBS during pathogenesis, positive to bacitracin , and positive to CAMP test is also used to differentiate (S.agalactiae) from other streptococcal species. In this instance, we have a positive result, indicating that the colony tested is S.agalactiae. cfb gene encoding by CAMP factor. Because the cfb gene is so prevalent in GBS strains, the CAMP test or PCR check for the cfb gene was often employed to distinguish GBS from other Streptococcus species. Molecular diagnosis of S. agalatiae by detection of (atr) gene all bacterial isolates (100%) had atr gene Figure (1). Detection atr gene is the high specificity test for GBS screening in pregnant women. It was found only in S. agalactiae and encodes for the amino acid glutamines transporter, which has a high degree of specificity for S. agalactiae. Because it is a housekeeping gene, the probability of mutation is low(Schörner et al., 2014).

DNA concentration and purity

Nanodrop Spectrophotometer used to measure the extracted DNA concentration and purity; the results showed that the concentration of was ranged from (44.11 – 207.261) ng/ µl, while the purity of extracted DNA from studied isolates ranges between (1.717 – 2.014).

MOLECULAR DETECTION OF VIRULENCE FACTORS AND ANTIBIOTIC RESISTANCE GENES

Detection of ermB gene

The prevalence of this gene was detected in all S.agalactiae isolates(100%). The percentage of ermB in our study was very high compared to another study in Iran, where the gene was present at a low rate (29.3%)(Ghamari et al., 2022). Another study in Iran was (70%)(Motallebirad et al., 2021). ermB is one of erm genes that encoded Ribosomal modification, which is associated with (cMLSB)co-resistance to MLSB antibiotics with high-level resistance to all MLSB antibiotics(Ghamari et al., 2022). As a result of the presence of ermB gene, this increases its resistance to erythromycin in particular. So All isolates were resistant to erythromycin.

Detection of tet gene

Twenty-one isolates (95.45%) had tet gene.The prevalence of tet gene in the USA was 85.1% (Metcalf et al., 2017). Tet genes give to bacteria resistance mechanisms, including tet(L), tet(K), tet(A), and tet(B), which encode efflux pump proteins, tet(M), which encodes 292 a ribosomal protection protein, and tet(U) whose product has an unknown mechanism(Li et al., 2020). Tet genes are present in diseases, opportunistic pathogens, and normal flora, which accounts for the high tet gene prevalence. The tetracycline resistance that is significantly increased in isolates carrying this gene is also a result of the antibiotic's widespread use as a cheap preventative measure for both human and animal illnesses. It is well known that bacteria often become resistant to widely used antibiotics (Mudzana, Mavenyengwa, & Gudza-Mugabe, 2021).

Detection of scpB gene

The results revealed that 21/22(95.45%) isolates had this gene.The results of our study were close to those of a study conducted in Egypt; the ratio was 100%(Abdallah et al., 2021). Another study in Zimbabwe (90.1%)(Mudzana, Mavenyengwa, & Gudza-Mugabe, 2021). A study done by Hadi (2020) found the percentage of scpB was (97.8%)(Abbas, 2020). Group B or scpB a surface-associated serine protease called scpB breaks down the neutrophil chemoattractant C5a to prevent complement activation and mediate bacterial adhesion to fibronectin. Cellular adhesion and invasion appear to be facilitated by the scpB gene capacity to bind fibronectin. Human epithelial cell invasion was prevented by scpB gene mutation (Shabayek & Spellerberg, 2018).

Detection of hly gene

Twenty-one isolates (95.45%) had hly gene with an end product size equal to 199 bp. Similar studies in some countries reported the hly gene among GBS, as it was (97.8%) in Zimbabwe. hly gene in S. agalactiae is a virulence factor that codes for hyaluronate lyase, The ability of the protein to cleave hyaluronan allows invasion and spread easier of GBS(Emaneini et al., 2016; Mudzana, Mavenyengwa, & Gudza-mugabe, 2021). Studies revealed that hly plays a significant role in producing proinflammatory cytokines(Campo et al., 2012). Furthermore, hly is a means of enhancing mobility across the body's tissues, and as an antigenic distinguish that prevents their being recognized by phagocytes of the immune system(Mahesh et al., 2012). as a result, hly is an important factor in the pathogenicity of GBS.

Detection of mefA gene

Twelve isolates (54.54%) had mefA gene with an end product size equal to 348 bp. This result was contrary to another result in Iran, which was (2.4%)(Ghamari et al., 2022). But in Taiwan, the prevalence of mefA gene was approximate to those of our study, which was 49.2% (Lo et al., 2015). mefA gene encodes M phenotype of MLSB phenotypes. The mefA gene is responsible for regulating the Drug efflux that results resistance to macrolides (Wang et al., 2015).

Detection of Rib gene

Only five isolates (22.72%) had Rib gene. On the contrary, in Egypt, the ratio was different from the percentage we obtained in this study; it was (78.26%)(Abdallah et al., 2021).
In another study in Poland, the presence of the Rib gene was 23%(Brzozowska et al., 2022). Rib (rib gene) is a surface protein that mediates adhesion to host cells(Bobadilla et al., 2021). Rib gene were more variable. These variations may result from various horizontal gene transfer capacities(Sadowy et al., 2010).

Detection of bca gene

The prevalence of this gene was detected in three S.agalactiae isolates(13.63%). Contrary to our findings, another study reports a high prevalence of bca (86.0%) (Mudzana, Mavenyengwa, & Gudza-Mugabe, 2021). bca also known as alpha/α antigens of the C protein, was the most prevalent, which may indicate a risk for invasive illness because the Cα protein aids in the invasion of the cervical epithelium(Bobadilla et al., 2021). This variation may be due to different CPA types for GBS isolates in these two studies.

Detection of bac gene

The results revealed that 0/22(0%) of isolates had this gene, which means all isolates have no bac gene.There are similar results found to our result in Italy(Skov Sørensen et al., 2010). An approximate study to our study is the prevalence of bac (11.6%)(Mudzana, Mavenyengwa, & Gudza-Mugabe, 2021). In another study, the presence of bac was (58.50%), bac gene coding surface proteins Cβ, it is one of the main surface structures of GBS involved in virulence, which

mediates adhesion to host cells (Bobadilla et al., 2021). The β-antigen does not appear to be a crucial component of GBS virulence; instead, in some strains, a different protein is linked to the same function. Due to that, The S. agalactiae strains may use other surface-associated proteins during the infectious process in pregnant women if the bac gene is not found in an isolate of s. agalactiae (Skov Sørensen et al., 2010).

Correlation between the different genes found in S.agalactiae

Nine distinct virulence gene profiles were identified and the virulence gene profiles hly, scpB, rib(18.18%), hly, scpB, bca (13.63%), hly, bca (13.63%), hly, scpB, bca, rib (9.09%), hly, bca, rib(9.09%), scpB, bca, rib (9.09%), hly, scpB, bca, rib, bac (0%), and hly, scpB, bca, bac (0%) were common among GBS isolates.

In another study in zimbabwe hly-scpB-bca-rib 37.2% and hly-scpB-bca 18.6% were common among GBS isolates but hly, scpB, bca, bac 9.3%, hly, scpB, bca, rib, bac 2.3%, hly, scpB, rib 14%, hly, bca, rib 7%, and hly, bca 2.3% (Mudzana, Mavenyengwa, & Gudza-Mugabe, 2021).

4 CONCLUSION

In current work, GBS colonization rate (about 12.15%) and the prevalence of this bacteria among pregnant women in Anbar of Iraq. The diversity of virulence factors genes frequency were obtained from the present study because S.agalactiae isolates produce various types of resistance factor against common antibiotics such as biofilm formation and efflux pump. The emergence of macrolides-resistant S.agalactiae strains with a frequency of ermB is 100% co-carriage. In addition to antibiotic susceptibility, the selection of antibiotic therapy needs to be based on laboratory confirmation tests detecting antibiotic resistance, which will help prevent the occurrence and spread of antibiotic-resistant strains.

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