Egyptian Journal of Medical Microbiology Volume 29 / No.3 / July 2020 87-96 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Rate of Carriage of *Streptococcus agalactiae* among Pregnant Women and Role of Some Virulence Genes

[1]Amal F. Makled, [1]Ahmed B. Mahmoud, [2]Said A. Saleh, [1]Sara G. Hegazy*, [1]Rasha G. Mostafa
[1]Department of Medical Microbiology and Immunology, Faculty of Medicine, Menoufia University, Egypt
[2]Department of Obstetrics and gynecology, Faculty of Medicine, Menoufia University, Egypt

Key words:
GBS (Group B Streptococci), Pregnant women, Antimicrobial susceptibility, Virulence genes

Corresponding Author:
Sara Gamal Hegazy
Department of Medical Microbiology & Immunology, Faculty of Medicine, Menoufia University, Egypt
Tel.: 01093060752
sara.ali.nasr@icloud.com

ABSTRACT

Background: *Although, GBS is a natural flora of the ano-rectal region, it may colonize vagina and many infants can be infected during the passage through the birth canal. It has emerged as a leading cause of neonatal infections and deaths.* ***Objectives:*** *To estimate the rate of recto-vaginal carriage of GBS among pregnant females, describe its antimicrobial susceptibility profile and to detect some of its virulence genes by multiplex PCR.* ***Methodology:*** *Vaginal, anorectal and neonatal throat swabs which were collected from two-hundred and fifty pregnant women were inoculated in Todd-Hewitt broth for 24 hours then inoculated on blood agar plates. Antimicrobial susceptibility testing for GBS isolates was done and its virulence genes (scpB, bca, rib and HvgA) were identified by PCR. Also, the relation between these virulence genes and antimicrobial susceptibility was studied.* ***Results:*** *Among 250 pregnant females, 36(14.4%) were identified as GBS carriers with exclusive vaginal and anorectal colonization rates of 4% and 10.4% respectively. All isolates were susceptible to penicillin, ampicillin, cefepime, cefotaxime, ceftriaxone, vancomycin and linezolid.* On the other hand, *19.4%, 80.6%, 44.4% and 13.9% of GBS isolates were resistant to each of erythromycin and azithromycin, tetracycline, levofloxacin and clindamycin* respectively. *ScpB, rib, and Hvg-A genes were identified in 100%, 69.4% and 33.3% of GBS isolates respectively. None of them had the bca gene.* ***Conclusion:*** *Screening for GBS colonization of pregnant females is recommended and determination of virulence and different surface proteins would be relevant for better diagnosis and further possible formulation of a vaccine.*

INTRODUCTION

Streptococcus agalactiae; GBS is a Gram-positive cocci, encapsulated and non-motile. It may colonize genital tract and/or rectal area in 10-30% of pregnant women without obvious symptoms [1]. Not only GBS is the leading cause of neonatal infections but also its colonization of pregnant females increases the risk of infection incidents, abortion, rupture of membranes, preterm labor and puerperal fever [2].

In neonates, GBS colonization is transferred from colonized mothers during birth in 15–50% of cases, Many diseases may occurr in those newborns such as septicemia, pneumonia, urinary infections, and meningitis with high mortality rate (10–20% of cases) [3].

There are many factors that control and influence GBS colonization rates such as socioeconomic, cultural, and demographic conditions of pregnant women as well as the methods used for its detection. Prenatal GBS screening is recommended by the Centers for Disease Control and Prevention (CDC) by means of specimens harvested from the vaginal introitus and perianal region from all the pregnant women between 35 and 37 weeks of gestation [4].

Group B Streptococcus has a variety of virulence factors that facilitate its ability to cause disease. Some of these factors are capsular polysaccharides, regulatory proteins, surface-localized proteins and toxins [5]. Surface proteins act as adhesins and may also be involved in the evasion of the immune system. They include Bca (α-subunit of C protein), Bac (β-subunit of C protein), Rib (resistant to proteases immunity), Alp2 (Cα-like protein 2), C5a peptidase (ScpB), laminin-binding surface protein (Lmb), fibrinogen-binding protein (FbsA), secreted fibrinogen-binding protein (FbsB), cell surface protease (Csp), and surface protein of GBS (Spb1). GBS also produces a range of toxins such as hemolysins, hyaluronidase (hylB) and superoxide dismutase (SodA) and CAMP factor (cfb) that promote pathogen entry into host cells. These putative virulence factors have been investigated as possible vaccine candidates because of their ability to elicit protective immunity against GBS infection [6]. Also, *Hvg A* (Hypervirulent GBS adhesion) had been found to be expressed in some GBS strains and these strains adhere more efficiently to blood brain barrier cells. *Hvg-A* is a critical virulent trait of GBS in the neonatal context and stands as a promising target for the

DOI: https://doi.org/10.51429/EJMM29312

development of novel diagnostic and antibacterial strategies [7].

Penicillin G administered intravenously is the drug of choice for intra-partum prophylaxis, but ampicillin, clindamycin and erythromycin are an acceptable alternative. Vancomycin should be used in pregnant women allergic to penicillin [8].

As a result of the severity, spread of GBS infection and the controversy in its reported incidence, this study was performed to assess the percentage of *Streptococcus agalactaie* colonization among pregnant women and their newborns. Also, to study the potential relationships between genes encoding putative virulence factors with GBS isolates and their antibiotic resistance patterns.

METHODOLOGY

Subjects and data:

This study was carried out at the Microbiology and Immunology Department, in collaboration with Obstetrics and Gynecology department, Menoufia University and Omooma Hospitals during the period from November 2018 to November 2019. A written informed consents were obtained from all participants. This study was approved by the Local Ethics Committee, Faculty of medicine, Menoufia University. Demographic characteristics, history of antenatal care, medical and obstetric complications during current and previous pregnancy were collected from the pregnant women and documented in special data sheets.

Samples collection and Identification of bacterial isolates:

A total of 500 swabs (250 high vaginal and 250 anorectal) were taken from pregnant women at 35–37 weeks of gestation and 250 throat swabs were taken from their newborns' immediately after birth [9]. All samples were cultured on different media (Oxoid, UK) and processed according to standardized microbiological methods. Also, they were incubated in Todd-Hewitt broth (Difco Laboratories, Detroit, Michigan) with 8 mg gentamycin to inhibit other micro-organisms and to obtain pure growth of GBS. Subculture was done aerobically at 35 C^0 onto blood agar plates for 24 h. GBS isolates were identified by their specific colony morphology, β-haemolysis on blood agar and confirmed by VITEK 2 System. The confirmed GBS isolates were maintained in Todd-Hewitl broth supplemented with 20% glycerol and stored frozen at -80 °c [10].

Antimicrobial susceptibility testing by Kirby-Bauer disk diffusion method:

All GBS isolates were tested against different antimicrobial agents. The tested antimicrobial agents included penicillin (P, 10 units), ampicillin (AMP, 10mg), cefepime (FEP, 30mg), cefotaxime (CTX, 30mg), ceftriaxone (CRO, 30 mg), vancomycin (VA, 30 mg), erythromycin (E, 15mg), azithromycin (AZM, 15 mg), tetracycline (TC, 30 mg), levofloxacin (LEV, 5mg), clindamycin (DA,2 mg), linezolid (LZD, 30mg).Results were interpreted according to the guidelines of CLSI, (2019).

Molecular detection of rib, scp, Hvg A and bca virulence genes by multiplex PCR.

DNA extraction

- Bacterial DNA was extracted from GBS isolates according to the instructions provided by the gene JET [tm] genomic DNA purification kit (Thermo fisher scientific, UK) [11].
- Primers (table 1) were shipped and received in a lyophilizeal state (Invitrones, thermofisher, UK). The volume of nuclease –free H_2O added to the lyophilized primer was determined by reading the number of nmol of primers in the tube and multiplied by 10 to make100u/l primer stock.
- Multiplex PCR program (1 cycle) was performed in a thermal cycler (Applied Biosystems, Singapore). It consisted of a 95°C for 10 min to activate the enzyme AmpliTaq Gold DNA polymerase, then cycling parameters Initial denaturation was done at 94°c for the *scpB* gene (1min.) and at 95°c for the *rib* gene, *bca* gene for 5 minutes each. Then, Annealing at 50 C° for 1 min., Extension then was done at 72°C ° for 1 minutes and final extension at 72°C for 5min.[11,12]. The amplified DNA products were electrophoresed using 2% agarose gel (Ferementas, Lithuania) stained with ethidium bromide (sigma, USA) and the bands were visualized and photographed (Samsung, WB30F, Korea)

Statistical analysis:

Computer SPSS program version 20 was used. The results were expressed as percent. Chi-square test was performed and was considered significant at p value <0.05 15.

Table 1: Primer sequences and amplicon size [12, 13]

Genes		Primer sequence	Amplicon size (bp)
Hvg-A	F R	GGTGCTAAAGAGCAAGCACT TCCTGGACTTCGTCTTTCCTCA	345
rib	F R	TGATACTTCACAGACGAAACAACG CATACTGAGCTTTTAAATCAGGTGA	296
bca	F R	TAACAGTTATGATACTTCACAGAC ACGACTTTCTTCCGTCCACTTAGG	535
scpB	F R	ACAACGGAAGGCGCTACTGTTC ACCTGGTGTTTGACCTGAACTA	255

RESULTS

Clinical data and microbial infections in the studied participants:

A total of 750 swabs were taken (250 high vaginal and 250 anorectal from female, and another 250 swabs from their babies).There was a significant difference between Ommoma and Menoufia University Hospitals regarding the positive cultures as 66.7%vs. 94%, 60% vs, 95% and 26,7%vs. 55% of vaginal, anorectal and neonatal swabs showed positive cultures respectively. All isolates from vaginal, rectal and neonatal specimens of all participants were displayed in table 2a, b and c. The vaginal and anorectal colonization rates of GBS were 10/250 isolates(4%), and 26 /250 isolates(10.4%) respectively, but no GBS strains were isolated from baby samples (Fig. 1). Mainly GBS colonized pregnant women from both hospitals aged below 30 years. Notebly, there was a significant difference between the two hospitals' females regarding socioeconomic standard, as 75% of Omooma hospital pregnant females were of moderate socioeconomic standard but 80% of Menoufia Hospital pregnant women had low @@ standard. Other demographic and clinical differences noted between GBS-positive participants admitted to Omooma and Menoufia university hospitals were recorded in table 3.

Table 2a: Culture and frequency of isolated organisms from vaginal specimens in Omooma and Menoufia hospitals

Vaginal specimens N=250	**Omooma (n = 150)**		**Menoufia (n = 100)**		Total	χ^2	P
	No.	**%**	**No.**	**%**			
No growth	**50**	33.3	6	6	56	25.789*	<0.001*
Growth	**100**	66.7	94	94	194		
Gram-positive cocci							
Staph aureus	6	4	7	7	13	1.095	0.295
CONS	3	2	2	2	5	0.0	FEp=1.000
GBS	4	2.7	6	6	10	1.736	FEp=0.205
Enterococcus fecalis	10	6.7	9	9	19	0.465	0.495
Total	23	15.4	24	24	47	2.952	0.086
Gram-positive bacilli							
Lactobacilli	26	17.3	16	16	42	0.076	0.782
Diphteroids	11	7.3	12	12	23	1.564	0.211
Total	37	24.6	28	28	65	0.347	0.556
Gram-negative bacilli							
E-coli	1	0.7	1	1	2	0.084	FEp=1.000
Klebseilla	–	–	–	–	–	–	–
Pseudomonas	1	0.7	1	1	2	0.084	FEp=1.000
Proteus	8	5.3	8	8	16	0.712	0.399
Total	10	6.7	10	10	20	0.906	0.341
Fungi							
Candida	30	20	32	32	62	4.633*	0.031*

Table 2b: Culture and frequency of isolated organisms from anorectal specimens in Omooma and Menoufia hospitals

Ano-rectal specimens N=250	**Omooma (n = 150)**		**Menoufia (n = 100)**		Total	χ^2	P
	No	**%**	**No**	**%**			
No growth	60	40	5	5	65	38.202*	<0.001*
Growth	90	60	95	95	185		
Gram-positive cocci							
Staph aureus	2	1.3	2	2	4	0.169	FEp=1.000
CONS	35	23.3	25	25	60	0.091	0.762
GBS	8	5.3	18	18	26	10.331*	0.001*
Enterococcus fecalis	13	8.7	14	14	27	1.772	0.183
Total	58	38.6	59	59	117	9.963*	0.002*
Gram-positive bacilli							
Lactobacilli	–	–	–	–	–	–	–
Diphteroids	13	8.7	15	15	28	2.420	0.120
Total	13	8.7	15	15	28	2.420	0.120
Gram-negative bacilli							
E-coli	10	6.7	10	10	20	0.906	0.341
Klebseilla	6	4	6	6	12	0.525	0.551
Pseudomonas	2	1.3	3	3	5	0.850	FEp=0.651
Proteus	1	0.7	2	2	3	0.900	FEp=0.566
Total	19	12.7	21	21	40	3.100	0.078
Fungi							
Candida	–	–	–	–	-	–	–

Table 2c: Culture and frequency of isolated organisms from neonatal specimens in Omooma and Menoufia hospitals

Neonatal specimens N=250	**Omooma (n = 150)**		**Menoufia (n = 100)**		Total	χ^2	P
	No	**%**	**No**	**%**			
No growth	110	73.3	45	45	155	20.444*	<0.001*
Growth	40	26.7	55	55	95		
Gram-positive cocci							
Staph aureus	3	2	4	4	7	0.882	FEp=0.442
CONS	12	8	14	14	26	2.318	0.128
Strept Viridans	10	6.7	16	16	26	5.609*	0.018*
Total	25	16.7	34	34	59	9.998*	0.002*
Gram-positive bacilli							
Diphteroids	15	10	21	21	36	5.890*	0.015*

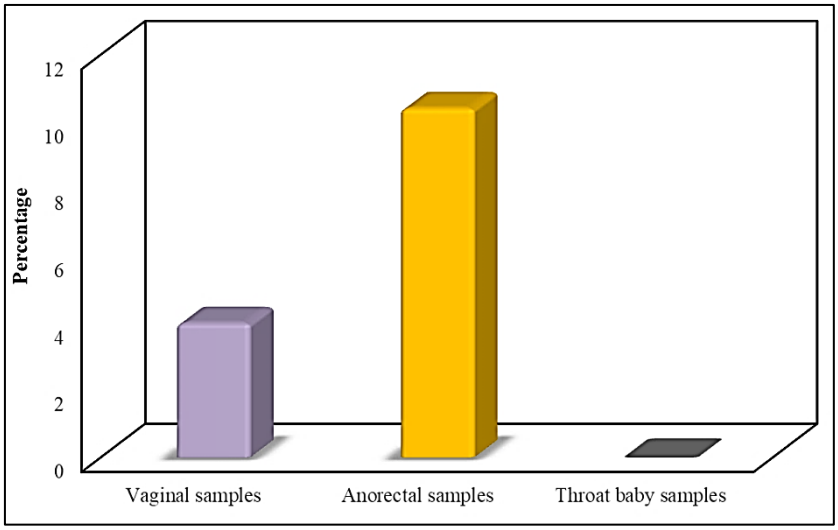

Fig. 1: Vaginal and anorectal colonization rate of GBS among pregnant women

Table 3: Demographic data and clinical characteristics of GBS female carriers from Omooma and Menoufia University Hospitals

Clinical characteristics	GBS +ve (n=36)				Total	χ^2	P
	Omooma (n=16)		Menoufia (n=20)				
	No.	%	No.	%			
Age in years							
<30	10	62.5	18	90	28	3.889	FEp=0.103
≥30	6	37.5	2	10	8		
Socioeconomic class							
Low	4	25	16	80	20	10.890*	0.001*
Moderate	12	75	4	20	16		
Contraception method							
No	10	62.5	10	50	20	1.383	MCp= 0.576
COC	1	6.3	4	20	5		
Loop	5	31.3	6	30	11		
Gravidity							
Primigravida	11	68.8	16	80	27	0.6	FEp=0.470
Multigravida	5	31.3	4	20	9		
Parity							
0	11	68.8	16	80	27	2.094	MCp=0.684
1	3	18.8	1	5	4		
2	1	6.3	1	5	2		
3	1	6.3	2	10	3		
Urinary tract infection							
No	5	31.3	2	10	7	2.563	0.204
Yes	11	68.8	18	90	29		
Vaginal douches used							
No	14	87.5	20	100.0	34	2.647	P=0.190
Yes	2	12.5	0	0.0	2		
Associated vaginal bleeding							
No	108	72.0	38	38.0	146	28.550*	<0.001*
Yes	42	28.0	62	62.0	104		
Vaginal discharge							
No	1	6.3	0	0	1	1.286	P=0.444
Yes	15	93.8	20	100	35		
Puerperal sepsis							
No	15	93.8	17	85	32	0.689	0.613
Yes	1	6.3	3	15	4		
Baby death							
Present	1	6.3	3	15.0	4	0.689	FEp=0.613
Absent	15	93.8	17	85.0	32		
Preterm delivery							
Present	9	56.3	9	45.0	18	0.450	0.502
Absent	7	43.8	11	55.0	18		
Neonatal sepsis							
Present	10	62.5	12	60.0	22	1.800	0.180
Absent	6	37.5	8	40.0	14		
PROM							
Present	5	31.3	11	55.0	17	2.031	0.154
Absent	11	68.8	9	45.0	20		

Antimicrobial susceptibility testing for the 36 GBS isolates:

Antimicrobial resistance pattern was tested for the 36 GBS isolates; none of the isolates was resistant to penicillins (penicillin and ampicillin), cephalosporins, vancomycin or linezolid. On the other hands, 7/36 (19.4%), 29/36 (80.6%), 16/36 (44.4%) and 5/36 (13.9%) of GBS isolates were resistant to erythromycin and azithromycin each, tetracycline, levofloxacin and clindamycin *respectively. Two isolates were intermediately sensitive to* **erythromycin,** tetracycline and clindamycin (Fig.2).

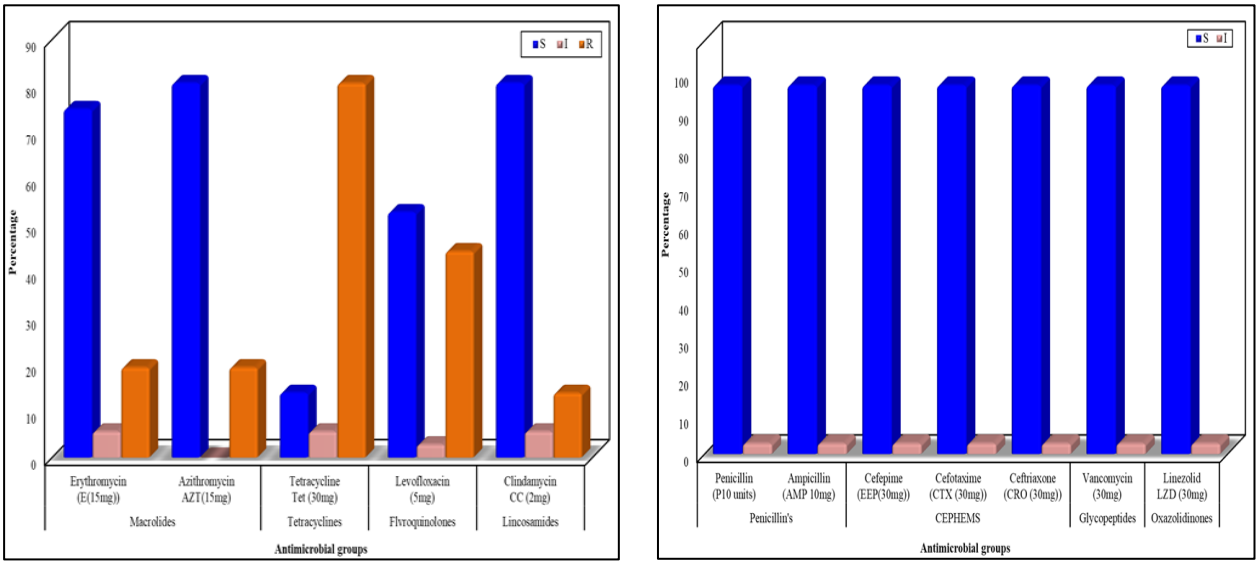

Fig. 2: Antimicrobial susceptibility pattern of GBS Isolates (n=36) by disk diffusion method

GBS virulence genes (rib, scp, Hvg A and bca) and their relation to antimicrobial susceptibility

The GBS isolates were examined by multiplex PCR for the presence of *scpB*, *Hvg-A*, *bca* and *rib* genes (Fig. 3). All isolates possessed *scpB* gene (100%) but no isolate harbored *bca* gene (0%). The existence of *rib and Hvg-A* genes gene were detected in 25/36 (69.4%) (17 anorectal and 8 vaginal isolates) and 12/36 (33.3%) isolates (5 anorectal and 7 vaginal isolates) respectively. (table 4). The association of GBS isolates' virulence genes with its resistance profile was studied (table 5); it showed that 44.4% of *scpB* positive GBS genotype was resistant to levofloxacin ،19.4% to each of erythromycin, azithromycin and 80.6% were tetracycline resistant isolates. Also، 75% and 50 % *Hvg-A* trait was tetracycline and levofloxacin resistant respectively, and 41.7% of them were resistant to erythromycin and azithromycin. While 80% of *rib* gene was detected as tetracycline resistant isolates, in 28% was levofloxacin resistant and 12 % was resistant to clindamycin and erythromycin

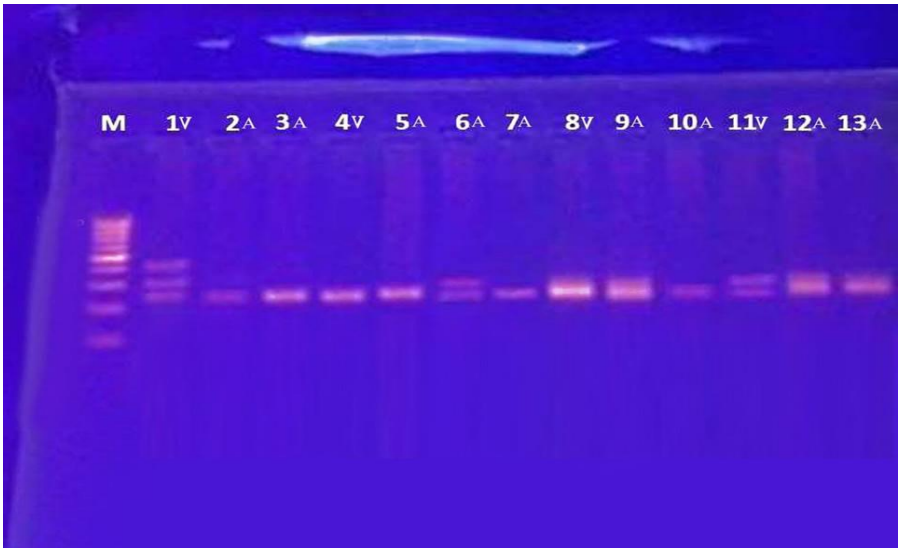

Fig (3): Agarose gel electrophoresis for the PCR amplified products of GBS *scpB, rib* and *HvgA* genes. (Lanes 2,3,5,6,7،9,10,12,13 were anorectal specimens, whereas Lanes 1,4,8,11 were vaginal specimens)
Lane (M) 100 bp DNA ladder. All lanes show *scpB gene at*255 bp. Lanes (1,6,11) show *rib* gene at 296 bp. Lanes (1) show *HvgA* gene at345 bp.
*V= vaginal *A= anorectal

Table 4: Frequency of detection of Rib, ScpB ,bca and HvgA genes in vaginal and anorectal specimens

Total cases (n=36)	*rib* gene				*Hvg A* gene				*scpB* gene				*bca* gene	
	Positive		Negative		Positive		Negative		Positive		Negative		positive	Negative
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No	%
Vaginal (N=10)	8	80	2	20	7	70	3	30	10	100	0	0	_	_
Anorectal (N=26)	17	65.3	9	34.7	5	19.2	19	80.8	26	100	0	0	_	_
Total	**25**	**69.4**	**11**	**30.6**	**12**	**33.3**	**24**	**66.7**	**36**	**100**	**0**	-	_	_

Table 5: Frequency of *rib, scpB, and HvgA* genes in relation to antimicrobial susceptibility by agar dilution method among 36 GBS isolates

Antimicrobial agents	*rib* gene				*scpB* gene				*Hvg A* gene			
	rib positive GBS. isolates (n=25)		*rib* negative GBS isolates (n=11)		*scpB* positive GBS. isolates (n=36)		*scpB* negative GBS isolates (0)		*Hvg A* positive GBS. isolates (n=12)		*Hvg A* negative GBS isolates (n=24)	
	n	%	N	%	n	%	n	%	n	%	N	%
Erythromycin												
Resistant	3	12	4	36.4	7	19.4	-	-	5	41.7	2	8.3
Sensitive	22	88	7	63.6	29	80.6			7	58.3	22	91.7
Azithromycin												
Resistant	2	8	5	45.5	7	19.4	-	-	5	41.7	2	8.3
Sensitive	23	92	6	54.5	29	80.6			7	58.3	22	91.7
Tetracycline												
Resistant	20	80	9	81.8	29	80.6	-	-	9	75	20	83.3
Sensitive	5	20	2	18.2	7	19.4			3	25	4	16.7
Levofloxacin												
Resistant	7	28	9	81.8	16	44.4	-	-	6	50	10	41.7
Sensitive	18	72	2	18.2	20	55.6			6	50	14	58.3
Clindamycin												
Resistant	3	12	2	18.2	5	13.9	-	-	4	33.3	1	4.1
Sensitive	22	88	9	81.8	31	86.1			8	66.7	23	95.9

X^2: Chi square test FE: Fisher Exact

P: p value for comparing between the studied groups

*: Statistically significant at $p \leq 0.05$

DISCUSSION

Nearly one in ten women presenting for labor was colonized with GBS, which represents a considerable burden of colonization within a context of the absence of maternal GBS routine screening and the frequency of maternal GBS colonization worldwide is 18% [14]. **So,** Screening of GBS colonization and antibiotic prophylaxis of colonized women is recommended for all pregnant females especially in situations associated with the risk of preterm delivery and premature rupture of membrane to reduce neonatal GBS infection [15].

In this study, the frequency of maternal GBS colonization was 14.4%. This percentage was fully consistent with Wali et al., [16] in Egypt (15.6%) but it was much higher than that recorded in other developing countries, such as India, Turkey and Kenya (2.3, 6.5, 12% respectively) [17]. On the other hand, the percentage of GBS colonization recorded in this study was very much lower than researches done by Shabayek et al.[18] (25.3%) in Egypt. All the reported data estimated the global maternal GBS prevalence are variable with substantial heterogeneity across and within regions due to many factors, including socio-economic factors, the availability of medical care, preventive services, varying clinical practice guidelines, different average child-bearing age, methods of samples collection and used techniques. Genetic factors may also contribute in these variations.[19]

Up tell now no established international standards for maternal sampling of GBS, however, CDC recommends recto-vaginal swabs at 35–37weeks and the use of selective enrichment broth culture, but this approach is not always possible [17]. In this study, anorectal and high vaginal swabs were taken from all female participants and colonization rates of GBS were 26/250 isolates (10.4 %, and 10/250 isolates; 4% respectively). Almost all of the researches were compatible with our research, such as Nkembe et al.[20] found that GBS vaginal colonization rate and rectal carriage percentage was 1% and 10% respectively. Also, Bidgani et al. [21] found that GBS prevalence in rectal area (30.7%) was higher than vagina colonization (27.7%). In contrast to this result, Orrett et al. [22] reported that the culture of vaginal samples yielded more GBS isolates compared to rectal area (65.2% vs 53.2%).

In this research, a comparison had been made between a private hospital (Omooma) and the other a public university (Menoufia University). There was a significant difference between the two hospitals regarding socioeconomic standard (25% vs.80%), preterm delivery (56.3 %vs.45%) and premature rupture of membrane (PROM) (31.3% vs 55%) of GBS females' carriers. In agreement with these results, Feikin et al., [23] reported a higher significant rate of PROM in pregnant women colonized with GBS, Valkenburg et al., [24] found that preterm labor was one of the complication of maternal GBS colonization. Intrapartum fever, PROM and preterm delivery are major risk factors for GBS colonization Werawatakul et al., [25].

Also, the majority of Omooma (68.8%) and Menoufia (90%) GBS colonized pregnant women had UTI in their current pregnancy. Meanwhile, a significant correlation between GBS colonization rate and UTI was reported by Lekala et al., [26]. In this study, pregnant women aged <30 years old were more susceptible to GBS colonization because this colonization rate is more in females with high sexual activity. These results were in agreement with Sadaka et al., [27].

The relationship between history of baby death or neonatal sepsis between Omooma and Menoufia GBS colonized pregnant was evident in this study, 1/16 (6.3%) and 3/20 (15%) of pregnant women carried GBS had a history infant death and 10/16 (62.5%), 12/20(60%) of them had a history of neonatal sepsis. On the other hand, Lekala et al., [26] reported that infant death and neonatal sepsis were not common complications in GBS colonized women.

In this study, none of the GBS isolates showed resistance to penicillin, ampicillin, cefepime, cefotaxime, ceftriaxone, vancomycin or linezolid. This was in agreement with Sadaka et al.,[27]. *On the other hand*, 19.4%, 80.6%, 44.4% and 13.9% of GBS isolates were resistant to each of erythromycin and azithromycin, tetracycline, levofloxacin and clindamycin *respectively*. Melo et al.[28] reported the same alarming resistance rates to tetracycline and levofloxacin**.** Khan et al.[29] recorded that beta-lactam drug the first and second line recommended drugs all are active against GBS strains**.**

In the present study, GBS isolates were screened for 4 genes (*rib*, *scpB*, *bca* and *HvgA*), the most commonly detected surface protein gene in GBS isolates was the *scpB* gene (100% of isolates). Similar result was published by Duarte et al.[30] as **t**he presence of the *scpB* gene in human isolates is mandatory**.** The *rib* gene was detected in 69.4% of GBS isolates as in Chukwu et al.[31] works. The Rib protein encoded by the *rib* gene has been found in a significant percentage in GBS strains that caused invasive neonatal infections [31].

In our study, none of the GBS isolates possed the *bca* gene, as many studies, *bca* and *rib* genes weren't present concomitantly in the GBS genome and only one of them was harbored by GBS as the Rib protein encoded by the *rib* gene and alpha c protein encoded by *bca* gene share several characters that suggested they may have a common origin [27]

In this study, 44.4% of *scpB* positive GBS genotype was resistant to levofloxacin ، 19.4% to each of erythromycin, azithromycin and 80.6% of them were tetracycline resistant isolates. Also, Sadaka et al.[27] demonstrated a significant correlation between antimicrobial resistance and genotype clusters denoting the distribution of particular clones with different antimicrobial resistance profile entailing the practice of caution in the therapeutic practices.

Conclusion and recommendation

It would appear that rectal specimens were more effective than vaginal ones in GBS detection. Intrapartum GBS genotype screening along with antimicrobial susceptibility profile and virulence encoding genes should be done to prevent GBS neonatal disease. Penicillin or ampicillin is still the antibiotic of choice for intrapartum prophylaxis. All isolates possessed *scpB* gene but no isolate harbored *bca* gene and *rib and Hvg-A* genes were detected in 69.4% and 33.3% respectively. *rib* gene was perceived in almost of tetracycline resistant GBS isolates. Moreover, further studies should be conducted to identify the most prevalent serotypes among Egyptian GBS isolates to guide in the design of appropriate vaccine.

Conflicts of interest:

The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

Funding: This research did not receive any specific grant from funding in the public, commercial, or not-for-profit sectors.

REFERENCES

1. Salloum M, Mee-Marquet N, Domelier A, Arnault L, Quentin R. Molecular characterization and prophage DNA contents of Streptococcus agalactiae strains isolated from adult skin and osteoarticular infections. J. Clinic. Microbiol. 2010; 48(4): 1261-1269.

2. Rausch AV, Gross A, Droz S, Bodmer T, Surbek DV. Group B Streptococcus colonization in pregnancy: Prevalence and prevention strategies of neonatal sepsis. J. Perinat. Med. 2009; 37: 124-129.
3. El Beitune P, Duarte G, Maffei CML. Colonization by Streptococcus agalactiae During Pregnancy: Maternal and Perinatal Prognosis. Br. J. Infec. Dis. 2005; 9(3): 276-282.
4. Konikkara KP, Baliga S, Shenoy SM, Bharati B. Comparison of various culture methods for isolation of group Streptococcus from intrapartum vaginal colonization. J Lab Physicians. 2013; 5:42-5.
5. Herbert MA, Beveridge Catriona JE. Bacterial virulence factors in neonatal sepsis: group B streptococcus. Curr Opin Infect Dis. 2004;17:225–229
6. Udo EE, Boswihi SS, Al-Sweih N. Genotypes and virulence genes in group B streptococcus isolated in the maternity hospital, Kuwait. Medical Principles and Practice. 2013;22(5):453-7
7. Khodaei F, Najafi M, Hasani A, Kalantar E, Sharifi E, Amini A. Pilus–encoding islets in S. agalactiae and its association with antibacterial resistance and serotype distribution. 2018;116:189–94. .
8. Schrag SJ, Zywicki S, Farley MM, Reingold AL, Harrison LH, Lefkowitz LB. Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. N Engl J Med. 2000; 342:15–20.
9. Al Hazzani AA, Bawazeer RA, Shehata AI. Epidemiological characterization of serotype group B Streptococci neonatal infections associated with interleukin-6 level as a sensitive parameter for the early diagnosis. Saudi Journal of Biological Sciences. 2018; 25(7):1356-64.
10. Campbell JR, Hillier SL, Krohn MA, Patricia F, Dori FZ, Carol JB. Group B streptococcal colonization and serotype-specific immunity in pregnant women at delivery. J. Obstet. Gynecol. 2000; 96: 498-503.
11. Sadeh M, Firouzi R, Derakhshandeh A, Khalili MB, Kong F, Kudinha T. Molecular characterization of Streptococcus agalactiae isolates from pregnant and non-pregnant women at Yazd University Hospital, Iran. Jundishapur journal of microbiology. 2016; 9(2).
12. Jain B, Tewari A, Bhandari BB, Jhala MK. Antibiotic resistance and virulence genes in Streptococcus agalactiae isolated from cases of bovine subclinical mastitis. Veterinarski arhiv. 2012; 82(5):423-32.
13. Tazi A, Disson O, Bellais S, Bouaboud A, Dmytruk N, Dramsi S, Mistou MY, Khun H, Mechler C, Tardieux I, Trieu-Cuot P. The surface protein HvgA mediates group B streptococcus hypervirulence and meningeal tropism in neonates. Journal of experimental medicine. 2010; 207(11):2313-22.
14. Kwatra G, Cunnington MC, Merrall E, Adrian PV, Ip M, Klugman KP. Prevalence of maternal colonization with Group B streptococcus: a systematic review and meta-analysis. Lancet Infect Dis. 2016;16(9):1076–1084.
15. Schuchat A, Whitney CG, Zangwill K. Prevention of perinatal group B streptococcal disease: a public health perspective. 1996; 45(31): 679.
16. Wali IE, Sorour AE, Abdalla MAH. (): Assessment of different methods for detection of Group B Streptococci carriage among pregnant females. Egypt J Med Microbiol. 2007;16: 593–598.
17. Gizachew M, Tiruneh M, Moges F, Adefris M, Tigabu Z, Tessema B. Streptococcus agalactiae from Ethiopian pregnant women; prevalence, associated factors and antimicrobial resistance: alarming for prophylaxis. Annals of clinical microbiology and antimicrobials. 2019;18 (1):3.
18. Shabayek S, Abdalla S, Abouzeid AM. Serotype and surface protein gene distribution of colonizing group B streptococcus in women in Egypt. Epidemiol Infect. 2014;142: 208–210.
19. Tash RM, Ahmed MI. (2019): Carriage of Streptococcus agalactiae among Pregnant Women in an Egyptian University Hospital, Serotypes Distribution and Antibiotics Susceptibility. 28(3):79-84.
20. Nkembe NM, Kamga HG, Baiye WA, Chafa AB, Njotang PN. Streptococcus agalactiae prevalence and antimicrobial susceptibility pattern in vaginal and anorectal swabs of pregnant women at a tertiary hospital in Cameroon. BMC Res Notes. 2018; 11:480.
21. Bidgani S, Navidifar T, Najafian M, Amin M. Comparison of Group B streptococci colonization in vaginal and rectal specimens by culture method and polymerase chain reaction technique. J Chin Med Assoc. 2016; 79(3):141–145.
22. Orrett FA.(2003): Colonization with Group B streptococci in pregnancy and outcome of infected neonates in Trinidad. Pediatrics international; 45(3):319-23.
23. Feikin DR, Thorsen P, Zywicki S. Association between colonization with group B streptococci during pregnancy and preterm delivery among Danish women. Am. J. Obstet. Gynecol., 2001; 184: 427-431.
24. Valkenburg-van den Berg AW, Houtman-Roelofsen RL, Oostvogel PM, Dekker PJ, Dörr PJ,

Sprij AJ. Timing of group B streptococcus screening in pregnancy: a systematic review. Gynecol Obstet Invest. 2010; 69:174–183.

25. Werawatakul Y, Wilailuckana C, Taksaphan S, Thinkumrup J, Pragarasung M, Chouwajaroen P, Wachirapakorn J, Kenprom M. Prevalence and risk factors of Streptococcus agalactiae (group B) colonization in mothers and neonatal contamination at Srinagarind Hospital. Journal of the Medical Association of Thailand= Chotmaihet thangphaet. 2001; 84(10):1422-9.
26. Lekala LM, Mavenyengwa RT, Moyo SR, Lebelo SL, Bolukaoto JY, Chukwu MO. Risk factors associated with Group B Streptococcus colonization and their effect on pregnancy outcome. J Gynecol Obstet. 2015; 3(6):121–8.
27. Sadaka SM, Aly HA, Meheissen MA, Orief YI, Arafa BM. Group B streptococcal carriage, antimicrobial susceptibility, and virulence related genes among pregnant women in Alexandria, Egypt. Alexandria J Med. 2018; 54:69–76.
28. Melo NC, Oliveira MD, Scodro RB, Cardoso RF, Pádua RA, Silva FT, Costa AB, Carvalho MD, Pelloso SM. Antimicrobial susceptibility of *Streptococcus agalactiae* isolated from pregnant women. Revista do Instituto de Medicina Tropical de São Paulo. 2016;58.
29. Khan MA, Faiz A, Ashshi AM. Maternal colonization of group B Streptococcus: prevalence, associated factors and antimicrobial resistance. Ann Saudi Med. 2015; 35:423–427.
30. Duarte RS, Bellei BC, Miranda OP. Distribution of antimicrobial resistance and virulence-related genes among Brazilian group B streptococci recovered from bovine and human sources. Antimicrob Agents Chemother. 2005; 49:97–103.
31. Chukwu MO, Mavenyengwa RT, Monyama CM, Bolukaoto JY, Lebelo SL, Maloba MR, Nchabeleng M, Moyo SR. (2015): Antigenic distribution of Streptococcus agalactiae isolates from pregnant women at Garankuwa hospital–South Africa. Germs; 5(4):125.