CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.3581189

Available online at: http://www.iajps.com *Research Article*

PREVALENCE AND ANTIBIOGRAM OF GROUP B STREPTOCOCCUS [GBS] ISOLATED FROM A FRESHWATER CATFISH [CLARIAS GARIEPINUS, BURCHELL 1822]

[1]Barika P.N., [2*]Akani, N.P.

[1,2] Department of Microbiology, Rivers State University, Nkpolu - Oroworukwo, Port Harcourt P.M.B. 5080. Rivers State Nigeria.

Article Received: October 2019 **Accepted:**November 2019 **Published:**December 2019

Abstract:

*This research was carried out to determine the antibiotic susceptibility pattern of Group B Streptococcus [Streptococcus agalactiae] isolated from freshwater catfish [Clarias gariepinus] to some conventional antibiotics. Thirty[**30] fish samples from five different locations were collected and analyzed using standard microbiological techniques. Twelve [12] S. agalactiae were isolated, the Total heterotrophic bacteria counts ranged from** 1.28±0.21x10^{7}cfu/g to 1.89±0.08 x10^{7}cfu/g in Wimpey and Rumuokoro respectively. There was a difference [p≤0.05] in the bacterial population between the different markets. The highest occurrence of S. agalactiae was recorded in Creek road and Mile 1 Markets [25%] and the least was observed in Ozouba, Rumuokoro and Wimpey [16.7%]. S. agalactiae was highly susceptible to Ciproflox [83.3%] followed by Tarivid [66.7%]. The susceptibility pattern is as shown in decreasing order of resistance: Nalidixic acid and Augumentin [100%]> Ampicillin [91.7%]>Septrin and Streptomycin [75.0%]>Ceporex and Gentamicin[58.3%]>Pefloxacin [33.3]. All Streptococcus[100%]had a MAR index > 0.2which indicates a high risk source of contamination and often use of antibiotics. Public health awareness campaigns are advocated in markets on safe and hygienic practices in the handling and distribution of C. gariepinus as well as the implication of GBS infection. Drug abuse and misuse should be highly discouraged to reduce the prevalence of resistant strains due to GBS infections.*

Keywords:*Streptococcus agalactiae,Clariasgariepinus, Prevalence, Antibiotic resistance*

Corresponding author:

Akani, N.P,

Department of Microbiology, Rivers State University,
Nkpolu - Oroworukwo, Port Harcourt P.M.B. 5080. Rivers State Nigeria.
Email: nedieakani@gmail.com

QR code

Please cite this article in press Akani, N.P et al., ***Prevalence And Antibiogram Of Group B Streptococcus [Gbs] Isolated From A Freshwater Catfish [Clarias Gariepinus, Burchell 1822].,*** *Indo Am. J. P. Sci, 2019; 06[12].*

INTRODUCTION:

Group B Streptococcus [GBS] is a type of gram-positive streptococcal bacteria also known as *Streptococcus agalactiae*[1]. *S. agalactiae* is a normal flora of the human body such as the gastrointestinal, genitourinary tract and in the gut of Catfish [*Clarias gariepinus*], a species of catfish of the family Clariidae, the air-breathing catsfish which inhabits freshwater, lakes, rivers and swamps [2]. However, in certain cases, it causes opportunistic infections that can affect non-pregnant adults, pregnant women, and their new-born infants [3]. For many years *S. agalactiae* has been a contagious pathogen from dairy cattle, especially food like the bovine milk that cause mastitis which affect milk quality and decrease in milk production, bovine feces and from the environment [Dairy farms].Most of the *S. agalactiae* isolated from the environment samples are very contagious[2]. It has been recorded that group B Streptococcus infection is associated with the consumption of raw fish dish leading to many food-borne infections [4]. In Nigeria, approximately 30,800 cases of invasive GBS disease occur annually across all age groups. Group B streptococcal disease is the most common cause of neonatal sepsis and meningitis in most countries [5]. The incidence of group B streptococcal disease in adults increases with age, with the highest rate in adults 65 years of age[6]. Although the incidence of neonatal group B strep infection has been decreasing, the incidence of GBS infection in non-pregnant adults has been increasing [7]. In 2000 to 2001, the reported overall incidence of GBS infection in new-born babies in some parts of Nigeria was 0.72 per 1,000 live births, 0.47 per 1,000 for GBS-EOD and 0.25 per 1,000 for GBS-LOD [1].It was reported in a study in Ile-Ife, western Nigeria that ninety-eight [98] GBS isolates were recovered from 170 pregnant women and their neonates [8]. Pregnant woman Age range 31-35 [31.8%] shows huge validity frequency for GBS followed by age range 26-30 [31.2%] and 41-45 [2.9%] which showed the lowest frequency among the patients [9]. GBS has numerous features that facilitate evasion of the host immune system and, thus, promote colonization and/or invasive disease. As is the case for many pathogenic streptococci, GBS produces a polysaccharide capsule that likely inhibits opsono-phagocytosis and dampens immune responses [10,11]. The capsule is the major target of host antibody responses, but the presence of 10 known GBS capsular types [Ia, Ib, II-IX] makes the development of widely cross-protective responses [either through natural infection or vaccination] challenging. Certain GBS serotypes, especially types Ia, Ib, II, III, and V, cause the majority of neonatal disease with symptoms such as fever, difficulty in feeding, sluggishness, difficulty in breathing, irritability and Jaundice [9].Direct targeting of human cells by GBS β-hemolysin/cytolysin, inhibition of oxidative killing by the GBS pigment, inactivation of complement components, and engagement of inhibitory immune receptors all contribute to the multipronged manipulation of human immunity by GBS [11]. Recent studies have also demonstrated the remarkable plasticity of the GBS genome, with transfer of large stretches of DNA driving GBS diversification over relatively short time periods [10]. The recognition that maternal colonization with the organism is a key factor in the occurrence of GBS-associated neonatal morbidity and mortality was a milestone in the history of perinatal health and also cause diseases in areas such as Urinary tract, Placenta Amniotic fluid, membrane lining of the uterus and bloodstream [11]. A nationwide change in health practices helped diminish mortality and morbidity associated with the disease. Group B Streptococcus [GBS] is a leading cause of neonatal infection in most path of the world [9]. The Group B *Streptococcus* has been known to be resistant to many antibiotics such as Ampicillin, Clidamycin, Augumentin and this resistance has been attributed to high use of drugs as a result of diseases caused by *Streptococcus* and they can also cause complications such as skin infection, Pneumonia, bone and joint infection, meningitis [11]. Hence, this research is aimed at determining the prevalence and antibiogram of Group B Streptococcus isolated from a freshwater catfish [*C .gariepinus* Burchell 1822] sold in Port Harcourt.

MATERIAL AND METHODS:

Description of study Area:

The study was carried out in five [5] different markets in Port Harcourt viz; Wimpey market, Creek road Market, Rumuokoro market, Ozuoba market and Mile 1 Market with coordinates 4.8273º N, 6.9820º E; 4.7583º N, 7.0209º E; 4.88472º N, 6.92667º E; 4.8713º N, 6.9299º E; 4.7918º N, 6.9986º E respectively.

Sample Collection:

A total of 30 samples of *C. gariepinus* were collected with sterile polythene bags from the five [5] different markets under hygienic condition in Port Harcourt Rivers State, and transported in an ice pack bag to the Department of Microbiology laboratory Rivets State University for further analyses.

Microbiological Analysis:

Bacteria Enumeration:

The enumeration of Total Heterotrophic bacteria count was carried out on Nutrient agar and the

isolation of *Streptococcus agalactiae* was done using blood agar [12]. The preparation of the stock analytical unit was done by weighing one[1] grams of the gut of the catfish after dissection was homogenized in 10ml of normal saline for bacterial enumeration, isolation and identification. Subsequent serial dilution was done by pipetting 1ml of the samples into 9ml of sterile normal saline for up to 6 dilutions. About 0.1 aliquot of the appropriate dilutions [10^{-5} and 10^{-6}] was inoculated in duplicates into already prepared sterile plates of Nutrient Agar and Blood agar using spread plate technique and incubated at 37ºC for 24hours [13]. Plates that grew between 30-300 colonies were counted and recorded.

Preservation of culture:
The pure cultures of the isolates was stored in 10% [v/v] glycerol suspension at -4ºC as a cryopreservative agent to prevent the damage of the pure cultures during drying for further analysis.

Isolation and identification of *Streptococcus agalactiae:*
Streptoccoccu sagalactiae was isolated based on its hemolytic activity [β-hemolysis] on blood agar using the streak plate method [12]. Biochemical test such as Gram staining, catalase, Coagulase, Oxidase, sugar fermentation, Methyl red, indole were carried out to confirm *S. agalactiae*[13]

Antibiotic Susceptibility Testing:
Ten commonly used antibiotic discs [µ/disc] which include: Pefloxacin[PEF]-10µg, Tarivid[OFX]-10µg, Ciproflox[CPX]-10µg, Augmentin[AU]-30µg, Gentamicin[CN]-10µg, Streptomycin[S]-30µg, Ceporex[CEP]-10µg, Nalidixic acid[NA]-30µg, Septrin[SXT]-30µg, Ampicillin[PN]-30µg were tested against the organism. The antimicrobial susceptibility profiles of the isolates where determined by Kirby-Bauer disk diffusion method [14] on sterile Mueller Hilton agar were the prepared bacteria suspension corresponding to 0.5McFarland Turbidity Standard was swabbed evenly on the plates with sterile swab sticks. The antibiotic disk were aseptically placed on the agar plates using sterile forcep after 3-5minutes and incubated at 35ºC for 24hours in an inverted position [15]. The zones of inhibition were measured and compared with CLSI [2017] standards.

Determination of Multiple Antibiotic Resistance [MAR] Index:
Multiple antibiotic resistance [MAR] index in relation to this study is referred to as the resistance of *S. agalactiae* isolate to three or more antibiotics [16]. Multiple antibiotic resistance [MAR] index was ascertained for each isolate by using the formula MAR = a/b, where a stands for the number of antibiotics to which the test isolate depicted resistance and b stands for the total number of antibiotics to which the test isolate has been evaluated for susceptibility [17].

Data Analysis:
Statistical Package for Social Sciences [SPSS] version 22 was used to analyze the data obtained from the study. The data was summarized using descriptive statistics for tabulation and representation. Analysis of Variance [ANOVA] was used to test for significant difference [$p \geq 0.05$] between the markets and where differences existed, Duncan Multiple Range Test was used to separate the means.

RESULTS:
The result of the bacterial population of the fish samples from the five different markets as presented in Table 1, showed that the total heterotrophic bacterial count ranged from 1.28±0.21 x 10^7cfu/g to 1.89±0.08 x 10^7cfu/g in Wimpey and Rumuokoro respectively. There was a difference [$p \leq 0.05$] in the bacterial population between the different markets.

Table 1: Total heterotrophic bacterial count in the samples [Catfish][x10^7cfu/g]

Location [Markets]	THBC
Creek Road	1.50 ± 0.10^{b}
Mile 1 Market	1.33 ± 0.03^{ab}
Ozuoba	1.46 ± 0.05^{ab}
Rumuokoro	1.89 ± 0.08^{c}
Wimpey	1.28 ± 0.21^{a}

*Means with same alphabet along the column shows no difference [$p \geq 0.05$]

Table 2: Frequency Distribution of *S. agalactiae* from Catfish intestine from Different Markets in Port Harcourt Rivers State Nigeria.

Sample Locations	Number	Percentage[%]
Creek road	3	25.0
Mile 1	3	25.0
Ozuoba	2	16.7
Rumuokoro	2	16.7
Wimpey	2	16.7
Total	**12**	**100**

The result as shown in Table 2 above indicate that creek road market and Mile 1 market has the highest occurrence [25%] while the least occurrence was observed in Rumuokoro, Ozuoba and Wimpey [16.7%].

Table 3: Susceptibility pattern of *S. agalactiae* from Catfish[*Clariasgariepinus*] intestines

Antibiotics	Concentration [µg]	Susceptible n[%]	Intermediate n[%]	Resistant n[%]
CPX	10	10[83.3]	2[16.7	0[0.00]
SXT	30	3[25.0]	0[0.00]	9[75.0]
S	30	3[25.0]	0[0.00]	9[75.0]
PN	30	0[0.00]	1[8.3]	11[91.7]
CEP	10	0[0.00]	5[41.7]	7[58.3]
OFX	10	8[66.7]	1[8.3]	3[25.0]
NA	30	0[0.00]	0[0.00]	12[100]
PEF	10	0[0.00]	8[66.7]	4[33.3]
CN	10	5[41.7]	0[0.00]	7[58.3]
AU	30	0[0.00]	0[0.00]	12[100]

PEF-Pefloxacin[10µg], OFX-Tarivid[10µg], CPX- Ciproflox[10µg], AU-Augmentin[30µg], CN-Gentamicin[10µg], S-Streptomycin[30µg], CEP-Ceporex[10µg], NA-Nalidixic acid[30µg], SXT-Septrin[30µg], PN-Ampicillin[30µg]

The result of the susceptibility test as shown in Table 3 revealed that the organism *S. agalactiae* where highly susceptible to Ciproflox [83.3%] followed by Tarivid [66.7%]. The susceptibility pattern is as shown in decreasing order of resistance: Nalidixic acid and Augumentin [100%]>Ampicillin[91.7%]>Septrin and Streptomycin [75.0%]>Ceporex and Gentamicin [58.3%]>Pefloxacin [33.3] and Tarivid [25.0%][Table 3].

Table 4: MAR Indices of *S. agalactiae*[N=12]

MAR INDEX	NUMBER [%]
0.4	2[16.7]
0.5	2[16.7]
0.6	2[16.7]
0.7	3 [25.0]
0.8	3 [25.0]

The result as shown in Table 4 above revealed that 100% of *S. agalactiae* has a MAR >0.2.

DISCUSSION:

Streptococcus agalactiae is a Group B Streptococcus [GBS] gram-positive streptococcal bacteria [1].It is encapsulated diplococcal organism exhibiting β hemolysis on blood agar and makes them more resistant to most antibiotics [3]. A total of twelve [12]*S. agalactiae* were isolated from the gut of catfish [*C.gariepinus*] from five different market in

Port Harcourt.Wimpey has the highest total heterotrophic bacteria count of 1.89±0.08 x10^7cfu/g and Rumuokoro had the least count 1.28±0.21x10^7cfu/g as in Table1. The high count is as a result of rigorous movement of the catfish within the market environment ,transportation and source of water, thereby exposing the catfish to microorganisms. Further, the handling process could lead to the distribution of GBS infections when these catfishes are consumed. The result is similar to report by [18].There was a difference in the total heterotrophic bacteria count between the markets which may be due to proximity of the markets and handling by customers who purchase the catfish.

The occurrence of *S. agalactiae* was high in Creek road market and Mile 1[25%] andOzuoba, Rumuokoro and Wimpey had the least occurrence of [16.7%] as shown in Table 2 and could be a result of several sanitary factors such as; poor cleaning and hand hygiene, undercooking, poor quality of raw materials, cross-contamination and poor temperature and time control [12].

The 12 isolates of *S. agalactiae* were subjected to ten [10] conventional antibiotics. *S. agalactiae* was susceptible to Ciproflox [83.3%] followed by Tarivid [66.7%]. The susceptibility pattern shows a decreasing order of resistance: Nalidixic acid and Augumentin [100%]>Ampicillin[91.7%]>Septrin and Streptomycin [75.0%]>Ceporex and Gentamicin [58.3%]>Pefloxacin [33.3]as shown in Table 3. The organism was totally resistant to Nalidixic acid and Augumentin [100%], this was also observed by [19] which shows that the organism were resistant to Nalidixic acid, Augmentin and Ampicillin and this can be explained by uncontrolled use of antibiotics in the treatment of GBS infections and the availability of these drugs non-restrictively in this areas which enables self-prescription. The 100% MAR> 0.2 explains this assertion. MAR index> 0.2 indicate a high use of antibiotic in that area and a high risk source of contamination and makes *S. agalactiae* resistant to many antibiotics[17].Further, the easy purchase over the counter might also be contributory causes for the selective pressure for the emergence of GBS resistance strain in the area [17].

CONCLUSION AND RECOMMENDATION:

This study recorded a high bacterial count in the gut of catfish and*S. agalactiae*was isolated which poses a serious public health threat when catfish contaminated with the organism is consumed. The organism was resistant to most of the antibiotics especially Nalidixic acidand Augmentin but generally were susceptible to Ciproflox and Tarivid which could be the drug of choice for the treatment of Group B Streptococcal infections acquired from undercooked *C. gariepinus*. The incessant use of antibiotics should be reduced, proper cooking and hygienic handling of food should be encouraged.

REFERENCES:

1. Lancefield, R. & Hare, R. [2003]. The serological differentiation of pathogenic and non-pathogenic strains of hemolytic streptococci from parturient women. *Journal of Medicine*, 61, 335-349.
2. Anoop, K.R., Sunda, K.S.G, Khan B.A & Las K.M. [2009]. Common Moorhen Gallinula chloropus in the diet of African catfish *Clarias gariepinus* in Keoladeo Ghana National Park, India. 5[2]: 22-23.
3. Doran, K. S & Nizet, V. [2014]. Molecular pathogenesis of neonatal group B streptococcalinfection: no longer in its infancy. *Molecular Microbiology*, 54[1], 23–31.
4. Mengist, A, Kanna, H., & Abdissa, A.[2016]. Prevalence and antimicrobial susceptibility pattern of Anorectal and vaginal group B Streptococci isolates among pregnant women in Jimma, Ethiopia BMC Res Notes9:351-353.
5. Tazi, A., Disson, O. & Bellais, S. [2010]. The surface protein HvgA mediates group B Streptococcus hypervirulence and meningeal tropism in neonates. *Journal of Medicine*, 207[11], 2313–2322
6. Fry, R. M. [2008]. "Fatal infections by haemolytic streptococcus group B.". *Lancet*, 231 [5969], 199–201.
7. Claeys, G, Verschraegen, G. & Temmerman, M. [2011]. "Modified Granada Agar Medium for the detection of group B streptococcus carriage in pregnant women". *Clinical Microbiology and Infection*, 7, 22–24.
8. Kwatra, G., Cunnington, M. C. & Merrall, E.[2016]. Prevalence of maternal colonisation with group B Streptococcus: a systematic review and meta-analysis. *Lancet of Infectious Disease*, 16[9], 1076–1084.
9. Hare, E. G, Rodríguez, M. C, Bartolomé, R., Berjano, B., Cabero, L. & Andreu, A. [2017]. "Evaluation of the Granada agar plate for detection of vaginal and rectal group B streptococci in pregnant women" [PDF]. *Journal of Clinical Microbiology*, 37 [8], 2648–2651.
10. Edmond, K. M, Kortsalioudaki, C. & Scott, S. [2012]. Group B streptococcal disease in infants aged younger than 3 months: systematic review and meta-analysis. *Lancet*, 379[9815], 547–556
11. Ferrieri, P., Cleary, P. P & Seeds, A. E. [2007]. Epidemiology of group B streptococcal carriage

in pregnant women and newborn infants. *Journal of Medical Microbiology*, 10, 103-114.
12. Assefa, A., Desta, K., & Lema, T. [2018]. Group B Streptococcal vaginal colonization and drug susceptibility pattern among pregnant women attending in selected public antenatal care centres in Addis Abab, Ethiopia BMC pregnancy Childbirth. 18:135-137.
13. Cheesbrough, M. [2006]. District laboratory practice in tropical countries, 2nd Part 2. Cambridge University Press, Cambride, UK. 62-70.
14. Bauer, A.W., Kirby, W.M., Sherris, J.C., and Jur, K.M. [1996]. Antibiotic Susceptibility testing by standard Single disc method. *Americal Journal of Clinical Pathology*. 5[10]: 496.
15. Clinical and Laboratory Standards Institute [2017]. Performance standards for antimicrobial Susceptibility testing. Nineteenth Informational Supplement. 2[3]:100-119.
16. Lambert, J.D., Hong, I., Lee, S.H., Shinko, R.J. & Yang, C.S. [2003]. Involvement of multidrug Resistance-associated proteins in regulating cellular levels of –Epigallocatechin-3-3 gallate and its methyl metabolites. Biochemical, Biophysioogy Res community 310[1]: 22-27.
17. Krumperman, P. H. [1985]. Multiple Antibiotic Indexing of E. coli to Identify High-Risk Sources of Fecal Contamination of Foods. *Applied and Environmental Microbiology* 46:165–170
18. Rachards, V.P., Lang, P., Bitar, P.D. Lefebure T.M., Schukken, Y.H., & Zadoks, R.N. [2011]. Comparative genomics and the role of lateral gene transfer in the evolution of bovine adapted *Streptococcus agalactiae*. Infection genetic Evolution. 11[12]: 63-75.
19. Yu, H., Lin, H., Yang, P., Hsu, C., Hsieh, W., & Tsao, L. [2011]. Group B Streptococcal infection in Taiwan: Maternal colonization and Neonatal infection. Pediatric Neonatology. 52:190-195.