PRINT ISSN 1119-8362
Electronic ISSN 2659-1499

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 29 (2) 581-586 Feb. 2025

Prevalence and Potential Risks of Antibiotic Resistance in *Streptococcus agalactiae* Isolates in Human and Non-Human Models Collected from Ituku-Ozalla, Enugu in South East Nigeria

***[1]OJINI, KI; [2]AINA, OS; [3]KOLAWOLE, RM; [4]ADEOYE, AR; [5]KUSHIMO, K**

*[*1]Department of Medical Laboratory Science, [2]Department of Biological Sciences, [5]Department of Biological Sciences, Trinity University, Lagos, Nigeria Lagos, Nigeria Lagos, Nigeria*
[3]Department of Cell Biology & Genetics, [4]Department of Cell Biology and Genetics, University of Lagos, Akoka, Lagos, Nigeria Trinity University, Lagos, Nigeria

**Corresponding Author Email: kelechi.ojini@trinityuniversity.edu.ng*
**ORCID: https://orcid.org/0009-0004-0856-1387*
**Tel: +234 803 0957 967*

Co-Authors Email: Oluwafemiaina100@gmail.com, rkolawole@unilag.edu.ng, Bioassaylab@yahoo.com, kolawolekushimo@gmail.com

ABSTRACT: *Streptococcus agalactiae*, a pathogen within the Streptococcaceae family, is significant for its role in neonatal infections, bovine mastitis, and zoonotic threats. Initially identified in animals, particularly in cases of bovine mastitis, *S. agalactiae* has also become a concern for human health, raising alarms about interspecies transmission, especially in regions with close human-animal contact. The extensive use of antibiotics in both human and veterinary medicine has accelerated the emergence of multi-drug-resistant (MDR) strains, complicating treatment efforts. Consequently, the objective of this paper was to investigate the prevalence and potential risks of antibiotic resistance in *S. agalactiae* isolates in human (pregnant and non-pregnant women) and non-human (cow's milk and fish tissue of catfish and Tilapia) models collected from Ituku-Ozalla, Enugu in South East Nigeria using appropriate standard procedures. Data obtained show that the percent (%) antibiotic resistance in human and non-human models were Ampicillin 55.6 and30; Penicillin 31.5 and 30; Erythromycin 27.8 and 35; Ceftriaxone 22.2 and 40; Chloramphenicol 46.3 and 20; Tetracycline 20.4 and 50 Gentamicin 42.6 and 40; Ofloxacin 25.9 and 35 and Ciprofloacin16.7 and 40 respectively. Significant antibiotic resistance was observed, particularly in human isolates against Ampicillin and Penicillin. On the other hand, non-human isolates exhibited higher resistance to Tetracycline, likely due to its widespread use in livestock. These findings indicate varying patterns of resistance between human and non-human isolates. The results emphasize the need for stricter antibiotic stewardship and surveillance to prevent the spread of MDR strains, especially in areas where human-animal interactions are common. Addressing these challenges is critical for public health, particularly in Nigeria, where the risks associated with interspecies transmission are pronounced.

DOI: https://dx.doi.org/10.4314/jasem.v29i2.29

Cite this Article as: OJINI, K. I; AINA, O; KOLAWOLE, R. M; ADEOYE, A. R; KUSHIMO, K (2025) Prevalence and Potential Risks of Antibiotic Resistance in *Streptococcus agalactiae* Isolates in Human and Non-Human Models Collected from Ituku-Ozalla, Enugu in South East Nigeria. *J. Appl. Sci. Environ. Manage.* 29 (2): 581-586

Dates: Received: 23 December 2024; Revised: 27 January 2025; Accepted: 09 February 2025; Published: 28 February 2025

Keywords: *Streptococcus agalactiae*; antibiotic; resistance; interspecies

The family Streptococcaceae consists of the genus Streptococcus, which is further categorized into alpha-hemolytic and beta-hemolytic groups based on hemolysis patterns on blood agar. Among the numerous species of Streptococcus, two groups, A and B, are particularly noteworthy due to their clinical relevance. *Streptococcus agalactiae*, belonging to Group B of the Lancefield classification,

**Corresponding Author Email: kelechi.ojini@trinityuniversity.edu.ng*
**ORCID: https://orcid.org/0009-0004-0856-1387*
**Tel: +234 803 0957 967*

is an opportunistic pathogen known for its role in neonatal infections, bovine mastitis, and emerging zoonotic threats (Lyhs *et al.*, 2016). *S. agalactiae* was initially identified as a pathogen in animals, specifically in cases of bovine mastitis. The bacterium's name, "agalactiae," meaning "no milk," reflects its detrimental impact on dairy production. Historically, the organism was confined to animal hosts, but subsequent studies revealed its ability to infect humans, particularly pregnant women, immunocompromised individuals, and neonates (Zadoks *et al.*,2011). The bacterium's adaptation to different hosts raises concerns about interspecies transmission, especially in regions where close human-animal interactions are common. The widespread use of antibiotics in both human and veterinary medicine has accelerated the development of antibiotic-resistant strains of *S. agalactiae*. This resistance complicates treatment protocols and poses a significant public health challenge. Recent studies have highlighted the emergence of multi-drug-resistant *S. agalactiae* strains, particularly in settings with inadequate antibiotic stewardship (Amundson *et al*.,2015). In recent years, a rapid increase in the incidence of various infections, including invasive infections due to GBS, has been reported. Between 1990 and 2017, a more than double increase in frequency was observed in all age groups, with the highest increase recorded in patients aged 65–79, with a mortality rate of up to 25%. The reasons for this trend have not been elucidated and vaccines are still under development. The primary reason is the rapidly developing multi-drug resistance (MDR) of GBS to at least three antimicrobial groups in recent years (Gergova *et al*., 2024). The objective of this paper is to investigate the prevalence and potential risks of antibiotic resistance in S. agalactiae isolates in humans (pregnant and non-pregnant women) and non-human (cow's milk and fish tissue of catfish and Tilapia) models collected from Ituku-Ozalla, Enugu South East Nigeria.

MATERIALS AND METHODS

Study Area and Sample Collection: This study was conducted in South East Nigeria, focusing on samples collected from the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu. A total of 599 samples were collected, comprising 399 human samples (205 high vaginal swabs and 194 urine samples) and 200 non-human samples (100 cow milk samples and 100 fish samples).

Human Samples: The human samples included high vaginal swabs (HVS) from 205 pregnant and non-pregnant women attending the obstetrics and gynecology clinic, and urine samples from 194 participants, including elderly men, diabetic patients, and individuals with compromised immune systems. Ethical clearance was obtained from the University of Nigeria Teaching Hospital Research Ethics Committee, and informed consent was secured from all participants.

Non-Human Samples: Non-human samples included milk from cows with clinical signs of mastitis and tissue samples from freshwater fish (Catfish and Tilapia) exhibiting symptoms consistent with streptococcal infections. The fish samples were purchased directly from fishermen in local markets and transported to the laboratory for analysis. The cow milk samples were obtained from local dairy farms and processed within 24 hours of collection.

L*aboratory Procedures - Culture and Isolation:* Human samples were cultured using Todd-Hewitt Broth (THB) supplemented with 1% yeast extract, 10 µg/ml colistin, and 15 µg/ml nalidixic acid. After incubation at 37°C in a 5% CO2-enriched environment for 18-24 hours, the enriched broths were sub-cultured onto Islam Agar GBS base (Oxoid) and blood agar plates. The plates were incubated under similar conditions for 18-24 hours. Non-human samples were processed by inoculating 10 µl of cow milk onto Trypticase Soy Agar (TSA) supplemented with 5% bovine blood. Fish tissues were homogenized, and a loopful of the homogenate was streaked onto TSA with 5% bovine blood. Both media were incubated at 37°C under microaerophilic conditions for 18-24 hours.

Biochemical Identification: Isolates were identified based on Gram staining, catalase testing, bile esculin hydrolysis, CAMP testing, and hippurate hydrolysis. Only isolates confirmed as *S. agalactiae* were subjected to further analysis.

Antibiotic Susceptibility Testing: Antibiotic susceptibility testing was performed using the disk diffusion method on Mueller-Hinton agar supplemented with 5% sheep blood.

The antibiotics tested included Ampicillin, Penicillin, Erythromycin, Ceftriaxone, Chloramphenicol, Tetracycline, Gentamicin, Ofloxacin, and Ciprofloxacin.

The zones of inhibition were measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. A zone of inhibition (ZOI) of 14 mm or more was considered indicative of sensitivity, while a ZOI less than 14 mm was classified as resistant.

RESULTS AND DISCUSSION

Antibiotic Resistance in Human Isolates: Significant resistance was observed in isolates from urine samples, particularly among elderly and diabetic patients. Resistance to Ampicillin and Penicillin was notably high, with over half of the isolates showing resistance to Ampicillin. This trend raises concerns about the efficacy of first-line antibiotics in treating *S. agalactiae* infections in this population. A total of 54 *S. agalactiae* isolates were obtained from human samples, with 20 isolates from HVS and 34 from urine samples. Ethical clearance was obtained from the University of Nigeria Teaching Hospital, Enugu State. The resistance patterns observed are shown in Figure 1.

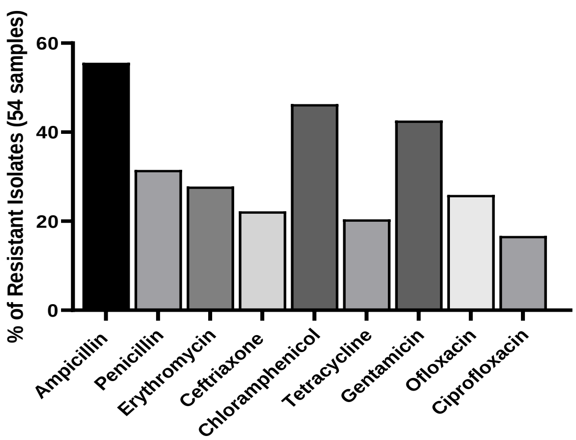

Fig 1: Percentage resistance isolates of 54 samples

Antibiotic Resistance in Non-Human Isolates: From the 200 non-human samples, 20 *S. agalactiae* isolates were obtained (10 from cow milk and 10 from fish tissues). Resistance to Tetracycline was notably higher in non-human isolates, particularly cow milk ones. The data suggest that the widespread use of Tetracycline in veterinary medicine may contribute to the development of resistant *S. agalactiae* strains. The resistance patterns are also shown in Figure 2.

Comparison of Human and Non-Human Isolates: A comparative analysis revealed that non-human isolates exhibited a slightly higher resistance rate to most antibiotics compared to human isolates. The non-human isolates showed a higher resistance to Tetracycline, which could be attributed to the extensive use of this antibiotic in livestock. The antibiotic resistance seen in the human isolates could be attributed to the fact that in this part of the world, antibiotics can be procured over the counter thereby creating room for indiscriminate use of antibiotics resulting in drug resistance.

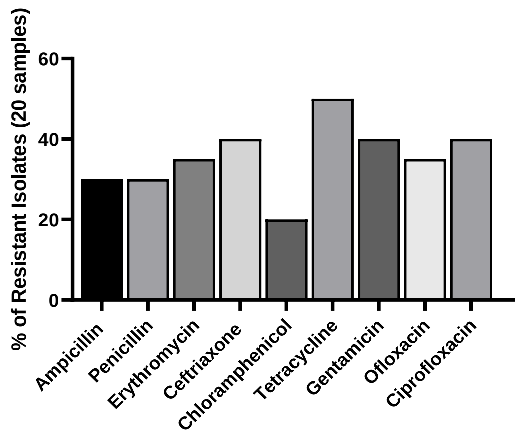

Fig 2: Percentage resistance isolates of 20 samples

Table 1: Summary of antibiotic sensitivity patterns for human and non-human isolates

Source	**Human**	**Non-Human**
No. Tested	54	20
Ampicillin (%)	55.6	30
Penicillin (%)	31.5	30
Erythromycin (%)	27.8	35
Ceftriaxone (%)	22.2	40
Chloramphenicol (%)	46.3	20
Tetracycline (%)	20.4	50
Gentamicin (%)	42.6	40
Ofloxacin (%)	25.9	35
Ciprofloxacin (%)	16.7	40

The findings of this study highlight the significant challenge posed by antibiotic-resistant *S. agalactiae* in South East Nigeria. The high resistance rates observed in both human and non-human isolates suggest widespread misuse or overuse of antibiotics in both medical and veterinary settings. Several medicines intensively used in the treatment of pathologies caused by GBS in animals, mainly in mastitis and freshwater fish, are also used in the treatment of human diseases. The high pressure of selection caused by the intensive and sometimes abusive use of antimicrobials contributes to the increase of antimicrobial resistance. This creates the risk of the inefficacy of the treatment protocols for animal and human infections caused by this pathogen. Furthermore, the occurrence of multidrug resistance (MDR) strains of GBS among animals, especially in fish and cattle, increases the threats to human health by the occurrence of interspecies infection, as pointed out in several studies (Botelho *et al.,* 2018) ; (Jaramillo-Jaramillo *et al.,* 2018) posed with the risk of horizontal transference of resistance genes among Group B Streptococcus of animal and human sources which highlights the need for surveillance over the emergence of MDR *S. agalactiae* among animal strains.

The resistance to Ampicillin and Penicillin in human isolates is particularly alarming, as these antibiotics are often used as first-line treatments for *S. agalactiae* infections. These drugs are

referred to as the Beta-lactam antibiotics. GBS is generally considered to be sensitive to β-lactams including penicillin which is the first-line drug for the prevention and treatment of GBS infection. (Seki.*et al*.,2015). However, GBS appears with decreased sensitivity to penicillin, Penicillin Resistant GBS (PRGBS). The first reports were from Japan. In addition, PRGBS has also been identified and reported in Canada and BUSA (Kusumademi *et al*., 2022). As earlier stated, antimicrobial resistance for both humans and non-humans occurs mainly through inappropriate drug prescriptions, such as overuse or overdose of antimicrobial drugs.

Bacteria can have resistance traits naturally (intrinsically resistant) and acquired (horizontal gene transfer). In general, the mechanism of antimicrobial resistance in bacteria can be divided into 1) limiting drug uptake; 2) modifying the drug target; 3) deactivating the drug, and 4) drug efflux (Reygaert *et al.,* 2018). β-lactam antibiotic resistance can occur through modification of the drug target and inactivation of the drug. Modification of drug targets is a mechanism of resistance to β-lactam antibiotics by changing the number and or structure of PBPs (penicillin-binding proteins). changes in the number of PBPs have an impact on the amount of drug that can bind to the target, while changes in structure cause a decrease in the drug's ability or even ability to bind to the target.

Another mechanism is to inactivate the drug, where this can be done by the transfer of chemical groups to the drug and degradation of the drug (β-lactamases). β-lactamases can inactivate β-lactam antibiotics by hydrolyzing a specific part of the β-lactam ring structure which makes the ring open so that the drug cannot bind to the target protein PBP (Reygaert *et al*., 2018). In this case, it is possible that the mechanism described above can cause resistance to β-lactam antibiotics. The high resistance rates observed in elderly and diabetic patients underscore the need for targeted antibiotic stewardship programs in these vulnerable populations. More Reports of decreased susceptibility emerged, which was stated by (Kanambath *et al.,* 2023) and was referred to as penicillin-resistant group B Streptococcus (PR-GBS). They stated that the most common mechanism of resistance detected by them was a mutation of the Penicillin-binding protein 2x gene (pbp2x gene). The lower resistance rates in non-human isolates may reflect differences in antibiotic exposure, but the high resistance to tetracycline in the non-human samples raises concerns about the potential for zoonotic transmission of resistant strains. However, antibiotic resistance to Gentamicin was observed in the human and non-human samples. Currently, the empiric antimicrobial therapy for sepsis and meningitis is the combined use of penicillin (or ampicillin) and gentamicin (Bradley *et al*., 2021) The bactericidal synergism is ineffective in the case of high-level gentamicin resistance (HLGR), due to acquired aminoglycoside-modifying enzymes. GBS is intrinsically resistant to low-level gentamicin, as was seen in this study, and surveillance screening schemes usually do not include gentamicin or other aminoglycoside susceptibility testing because no recommendations or breakpoints for testing HLGR are present in the guidelines issued by the Clinical and Laboratory Standards Institute (Creti *et al*., 2024)

The study also highlights the potential role of veterinary medicine in the development of antibiotic resistance in *S. agalactiae*. The extensive use of Tetracycline in livestock is likely contributing to the high resistance rates observed in cow milk isolates. This finding underscores the importance of implementing strict regulations on antibiotic use in agriculture to prevent the spread of resistant bacteria from animals to humans.

The study's findings are consistent with previous research conducted in other parts of Nigeria and Sub-Saharan Africa, which has documented high levels of antibiotic resistance in both human and veterinary settings (Oni *et al*., 2020). Zakerifar (2023) reported a pattern of antimicrobial resistance of isolates from pregnant women, among the investigated antibiotics, the highest resistance rates were found against tetracycline (94.33%), ofloxacin (78.3%), levofloxacin (67.92%), erythromycin (67.92%), and quinupristin/dalfopristin (60.37%), respectively. However, this study is one of the few to directly compare resistance patterns in *S. agalactiae* isolates from human and non-human sources, providing valuable insights into the potential for interspecies transmission of resistant strains.

The tetracycline genetic resistance determinants in Streptococcus are constituted principally by six resistance genes (tetK, tetL, tetM, tetO, tetQ, and tetT) characterized by two action mechanisms: the genes coding for efflux proteins and those coding for ribosomal protection enzymes (Haenni *et al.,* 2018).

These resistance genes have been described in GBS isolated from bovine mastitis (Silva *et al*., 2017). Although these genes were not analyzed in this work, this may explain the resistance levels to tetracycline observed in this study.

The use of antimicrobials is indispensable to protect human and animal health and animal welfare. However, the incorrect use of medicines worldwide has contributed to the increase of the resistance of pathogens involved in human and animal diseases. This poses a threat to controlling bacterial diseases in humans and animals throughout the world (World Organization for Animal Health, 2012). It is important to emphasize that, according to the World Health Organization (WHO), resistance is currently one of the greatest public health problems worldwide, putting at risk mainly immunodeficient individuals, such as neonates, pregnant women, and the elderly. As such, resistance requires the WHO's permanent monitoring, as well as the dissemination of its knowledge and implications for collective health (da Costa *et al*., 2021).

The study's limitations include its relatively small sample size and the focus on a single geographic region. Future research should aim to include a larger and more diverse sample population to provide a more comprehensive understanding of the prevalence and patterns of antibiotic resistance in *S. agalactiae* in Nigeria. Additionally, molecular typing of the isolates would provide further insights into the mechanisms of resistance and the potential for clonal spread of resistant strains across different hosts.

Conclusion: This study underscores the significant public health threat posed by antibiotic-resistant *Streptococcus agalactiae* in South East Nigeria. The findings reveal alarming resistance rates in both human and non-human isolates, particularly to first-line antibiotics like Ampicillin and Penicillin, which complicates treatment efforts. The higher resistance to Tetracycline in non-human samples, likely due to its extensive use in livestock, further highlights the risk of zoonotic transmission. These results emphasize the urgent need for targeted antibiotic stewardship programs, stricter regulations on antibiotic use in agriculture, and enhanced surveillance to mitigate the spread of multi-drug-resistant strains across species.

Declaration of Conflict of Interest: The authors declare no conflict of interest.

Data Availability: Data are available upon request from the first author.

REFERENCES

Amundson, MB; Seiler, CA; Jones, RN (2012). Emerging Multi-Drug Resistance in *Streptococcus agalactiae* from Human and Veterinary Samples. *J Infect Dis* 2015; 212(4): 635-642.

Botelho, CAN; Ferreira, AFM; Fracalanzza, SEL; Teixeira, LM; Pinto, TCA (2018). A Perspective on the Potential Zoonotic Role of *Streptococcus agalactiae*: Searching for a Missing Link in Alternative Transmission Routes. *Front Microbiol*.9 1-5 http://dx.doi.org/10.3389/fmicb.2018.00608.

Bradley, JS; Barnett, ED; Cantey, JB (2021). Nelson's Pediatric Antimicrobial Therapy. 27th (Ed). American Academy of Pediatrics.

Creti, R; Imperi, M; Berahi, A; Gheradi, G (2024). Emergence of High-Level Gentamicin Resistance in *Streptococcus agalactiae* Hypervirulent Serotype IV ST1010 (CC452) Strains by Acquisition of a Novel Integrative and Conjugative Element. *Antibio*. 13(6): 491.DOI: https://doi.org/10.3390/antibiotics13060491

da Costa, GM; Ribeiro, NA; Gonçalves, MS; da Silva, JR; Custódio, DA; Mian, GF (2021). Antimicrobial Susceptibility Profile of *Streptococcus agalactiae* Strains Isolated from Bovine Mastitis. *Brazil J Vet Res Ani Sci*. 3;58:e178109 DOI:https://doi.org/10.11606/issn.1678-4456.bjvras.2021.178109

Gergova, R; Boyanov, V,;Muhtarova, A, Alexandrova, A (2024). A Review of the Impact of Streptococcal Infection and Antimicrobial Resistance on Human Health. *Antibio*.13(4): 360 DOI: https://doi.org/10.3390/antibiotics11113040360

Haenni, M; Lupo, A; Madec, JY (2018). Antimicrobial Resistance in Streptococcus spp. In: Schwarz, S; Cavaco, LM; Shen, J 9(Ed). Antimicrobial Resistance in Bacteria from Livestock and Companion Animals. ASM Press pp 159-184.

Jaramillo-Jaramillo, AS; Cobo-Ángel, CG; Moreno-Tolosa,Y; Ceballos-Márquez, A(2018). Antimicrobial Resistance of *Streptococcus agalactiae* of Human and Bovine Origin. *Med Vet Zoot*. 13(1): 62-79

Kanambath, R; Vasanthaiah, S; Jamir, I; Mandel, J (2023). The Trends of Isolation and Antimicrobial Susceptibility of Group B Streptococcus in Urine Culture: a 7-Year Cross-Sectional Study. *Int J Reprod Contracept Obstet Gynecol* . 12(12): 1784-1788.

Kusumadewi, YP; Ganes, AM; Tazkiya, I; Nuryastuti, N (2022). *Streptococcus agalactiae* is Resistant to Beta-Lactam Antibiotics in a Diabetic Patient with Foot Infection: A Case Report. *J Clin Microbiol Infect Dis*. 2(1)1-5 .DOI:http://doi.org/10.51559/jcmicl.v2il.13

Lyhs, U; Kulkas, H; Katholm, J; Zadok, R (2016). *Streptococcus agalactiae* Serotype IV in Human and Cattle, Northern Europe. *Emerg.Infec. Dis*.(12) 2097 -2103. DOI:https/doi.org/10.3201/eid 2212.151447

Oni, OA; Oladokun, A; Oyinloye, TB (2020). Antibiotic Resistance in Nigeria: A Systematic Review and Meta-Analysis. *Afr J Clin Microbiol*. 20(1): 23-34.

Reygaert, WC (2018). An Overview of the Antimicrobial Resistance Mechanisms of Bacteria. *AIMS Microbiol* . 4(3): 482-500.DOI: https/doi.org/10.3934/microbiol.2018.482

Seki, T; Kimura, K; Reid, ME; Miyazaki, A; Banno, H; Jin, W (2015). High Isolation Rate of MDR Group B Streptococci with Reduced Penicillin Susceptibility in Japan. *J. Infect Antimicrob Agents Chemother*. 70(10): 2725-2728.DOI: https://doi.org/10:1093/jac/dkv203

Silva, JR; Castro, GDA; Gonçalves, MS; Custódio, DAD; Mian, GF; Costa, GM (2017). In Vitro Antimicrobial Susceptibility and Genetic Resistance Determinants of *Streptococcus agalactiae* Isolated from Mastitic Cows in Brazilian Dairy Herds. *Sem.: Ciên. Agrá*. 38(4): 2581-2594. DOI:10.5433/1679 -0359.2017v38n4Supl 1p2581

World Organization for Animal Health. Antimicrobial resistance. Paris, France: OIE; 2021. Retrieved February 28, 2021, from https://www.oie.int/en/for-the-media/amr/

Zadoks, RN; Schukken, YH; Wiedmann, M (2011). Multilocus Sequence Typing of *Streptococcus agalactiae* from Bovine and Human Sources. *J Clin Microbiol*. 49(11): 3846-3851.DOI: http://doi.org/10.1128/JC.00579 -11

"Zakerifar, M; Kaboosi H; Goli, HR; Rahmani, Z; Ghadikolaii, FP (2023). Antibiotic Resistance genes and molecular typing of *Streptococcus agalactiae* isolated from pregnant women. *BMC Preg. Childbirth*; 23:43. https://doi.org/10.1186/s12884-023-05380-4