Alarming levels of Multidrug Resistance in aerobic gram-negative bacilli isolated from the nasopharynx of healthy under-five children in Accra, Ghana

MARY-MAGDALENE OSEI

Research Assistant
University of Ghana Medical School
Department of Medical Microbiology

31st August 2022
• Nasopharyngeal bacterial pathogens include both Gram-positive bacteria and Gram-negative bacteria (Zar and Ferkol, 2014).

• And in children, this may lead to the development of lower respiratory tract infections including pneumonia and bronchiolitis later in life (Vising et al., 2013).

• Children with nasopharyngeal carriage serve as reservoirs and transmitters of pathogens including antimicrobial resistant producing genes (Simell et al., 2014).
• Previous studies from Brazil, Indonesia, Angola and the Netherlands report a Gram negative bacilli (GNB) carriage prevalence varying from 5% to 57% in healthy children (Lima et al., 2010; Vissing et al., 2013; Farida et al., 2013; Wolf et al., 2001 and Wolf et al., 1999).

• There is no published evidence on nasopharyngeal carriage of aerobic GNB from Ghana.

• The information is crucial to help in understanding the common aerobic GNB prevalent in Ghana and also serve as a baseline for the monitoring of future trends.
• This study was among healthy under-five children attending selected day-care centres in the Accra metropolis of the Greater Accra region of Ghana from September to December 2016, to...

(i) determine the prevalence of nasopharyngeal colonization of GNB and

(ii) describe the common organisms isolated and their antimicrobial resistance patterns including multidrug resistance (MDR), Extended-Spectrum Beta Lactamase (ESBL)-, AmpC- and Carbapenemase-producing bacilli.
Materials and Method

• Retrospective cross-section study involving frozen samples at -80°C in STGG collected as part of previous study conducted in 2016.

• Random sampling was made from seven day-care centres from four districts in the Accra metropolis.

Inclusion criteria: Pneumococcal vaccination

Exclusion criteria: No approved consent
Children who declined
Children with active upper respiratory tract infection
Antibiotic use
Achieved swabs in STGG

Plated on sterile MacConkey. Incubated for 18-24 hrs at 35 ±2 °C

GNB identification using MALDI-TOF after BTS passes

0.5 MacFarland using Phoenix nephelometer after calibrating with a standard

Antibiotic zones of inhibition were interpreted using CLSI 2021 version

AST
1. ESBL
2. AmpC
3. Carbapenemase

All antibiotics were controlled using ATCC E. coli 25922 control strain

Inoculated on Mueller Hinton to obtain confluent growth

Data analysis using STATA version 14.1
• Antibiotics selected for the research was based on

  • Ghana National Drugs Programme (GNDP) Standard Treatment Guidelines Republic of Ghana Ministry of Health.

  • WHO AWaRE Antibiotics 2021 AWaRe Classification.
The nasopharyngeal carriage prevalence of aerobic GNB was 14%, n = 57/410% (95% CI: 10.8%-17.6%).

| species                        | Prevalence                |
|-------------------------------|---------------------------|
| **Enterobacterales**          |                           |
| *E. coli*                     | (26.3%, n = 15/57);       |
| *Klebsiella pneumoniae*       | (24.5%, n= 14/57);        |
| *Enterobacter cloacae*        | (17.7%, n = 10/57)        |
| *Serratia marcescens*         | 1.8%, 1/57)               |
| **Acinetobacter baumannii**   | 8.9%, n = 5/57            |
| **Pseudomonas aeruginosa**    | 7.0%, n = 4/57            |
• Resistance was most frequently observed for cefuroxime (73.7%) followed by ampicillin (64.9%) and amoxicillin/clavulanic acid (59.6%).

• The organisms were least resistant to gentamicin (7.0%), amikacin and meropenem (both at 8.8%).

• Overall, MDR was observed in 66.7% (95% CI: 53.3%-77.8%) of isolates.

• MDR was relatively higher in Acinetobacter baumannii (100%), E. cloacae (90%) and E. coli (80.0%).
## Results Cont’d

| Species            | AmpC  | ESBL  | Carbapenemase |
|--------------------|-------|-------|---------------|
| **Enterobacterales** |       |       |               |
| *E. coli*          | 26.7% | 33.3% | 10.5%         |
| *K. Pneumoniae*    | 28.8% | 21.4% | 20.0%         |
| *A. baumannii*     | 100%  | 20%   | 20.0%         |
| *P. aeruginosa*    | -     | -     | -             |
• This is the first study from Ghana reporting on the prevalence of nasopharyngeal carriage of aerobic GNB and their resistance patterns in healthy under-five children.

• Many studies globally on nasopharyngeal carriage were on Gram-positive bacteria and anaerobic Gram-negative bacteria, however evidence on aerobic GNB is limited.

• Consequently, this study also contributes to the limited global evidence on this issue.
• One in seven children were carriers of aerobic GNB. Prevalence of aerobic GNB carriage was 14% in our study.

• *E.coli*, *K. pneumoniae* and *E. cloacae* were the commonest organisms and accounted for two-thirds of all organisms isolated.

• Resistance levels were high and two-thirds of the organisms exhibited MDR.
Conclusions

• In this first-ever study from Ghana calls for a nationwide surveillance system with data collected periodically;

  • to help in generating a national representative information which can be used to inform the choice of antibiotics in empiric treatment of infections caused by GNB.

• The evidence also calls for better infection prevention and control at the day-care centres in Ghana to prevent further transmission.
Limitations

• Overall, with the prevalence of GNB carriage in the healthy under-five children, the sample size was not sufficient to estimate the resistance levels in individual bacteria.

• The study was conducted in one of the areas of Accra and, thus, we feel that the findings are not generalizable nationwide.
The study used samples collected in 2016, this reflects the situation six years ago;
  • This warrants a follow-up study to assess the current rates of nasopharyngeal carriage and resistance levels.

A single study from one city may not be representative of the situation in Ghana;
  • This calls for either a nationwide study or strengthening surveillance systems to routinely capture the GNB carriage rates in healthy children.
• Setting up sentinel sites for collection of nasopharyngeal samples on a periodic basis and analysed using molecular technology to know the circulating genes responsible for antimicrobial resistance.

• Setting up prospective follow-up studies to find out the factors associated with progression from carriage to infection.

• The high levels of GNB carriage and MDR call for improved infection prevention and control in day-care centres to prevent any further transmission.
Way Forward in AMR Fight

• The study is in line with strategic plan objectives of the Ghana National Action Plan on AMR;

  • To develop and implement infection prevention and control (IPC) policies and interventions in all relevant sectors nationwide.
  
  • To set research agenda into AMR in affected sectors.

  • To establish a surveillance system for antimicrobial resistance.

  • Increase national awareness of AMR.
• The WHO Structured Operational Research and Training IniTiative (SORT IT) through this research will help to formulate IPC policy;

• Specifically to target day-care centres or early child development centres.
• Zar, H.J.; Ferkol, T.W. The Global Burden of Respiratory Disease—Impact on Child Health. Pediatric Pulmonology 2014, 49, 430–434, doi:10.1002/ppul.23030.

• Vissing, N.H.; Chawes, B.L.K.; Bisgaard, H. Increased Risk of Pneumonia and Bronchiolitis after Bacterial Colonization of the Airways as Neonates. American Journal of Respiratory and Critical Care Medicine 2013, doi:10.1164/rccm.201302-0215OC.

• Simell, B.; Auranen, K.; Käyhty, H.; Goldblatt, D.; Dagan, R.; O’Brien, K.L.; Group (PneumoCarr), for the P.C. The Fundamental Link between Pneumococcal Carriage and Disease. Expert Review of Vaccines 2014, doi:10.1586/erv.12.53.
• Lima, A.B.M.; Leão, L.S.N. de O.; Oliveira, L.S. da C.; Pimenta, F.C. Nasopharyngeal Gram-Negative Bacilli Colonization in Brazilian Children Attending Day-Care Centers. Braz. J. Microbiol. 2010, 41, 24–27, doi:10.1590/S1517-83822010000100005.

• Farida, H.; Severin, J.A.; Gasem, M.H.; Keuter, M.; van den Broek, P.; Hermans, P.W.M.; Wahyono, H.; Ver-brugh, H.A. Nasopharyngeal Carriage of Klebsiella Pneumoniae and Other Gram-Negative Bacilli in Pneumonia-Prone Age Groups in Semarang, Indonesia. J Clin Microbiol 2013, 51, 1614–1616, doi:10.1128/JCM.00589-13.
• Wolf, B.; Rey, L.C.; Moreira, L.B.; Milatovic, D.; Fleer, A.; Verhoef, J.; Roord, J. J. Carriage of Gram-Negative Bacilli in Young Brazilian Children with Community-Acquired Pneumonia. International Journal of Infectious Diseases 2001, 5, 155–159, doi:10.1016/S1201-9712(01)90091-8.

• Wolf, B.; Gama, A.; Rey, L.; Fonseca, W.; Roord, J.; Fleer, A.; Verhoef, J. Striking Differences in the Nasopharyngeal Flora of Healthy Angolan, Brazilian and Dutch Children Less than 5 Years Old. Annals of Tropical Paediatrics 1999, doi:10.1080/02724939992383.
ANTIMICROBIAL RESISTANCE (AMR) COMMUNITY OF PRACTICE (CoP)

THANK YOU