

# BACTERIAL GASTROENTERITIS 2006

*Paradise Suites Hotel, Kololi, The Gambia*

*Monday 8th to Wednesday 10th, 2006*

## List of Participants

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### **Running Order of Oral Presentations**

1st – John Wain (1)	7th – Aaron White	13th – Deanna Gibson
2nd – Sharon Perry (1)	8th – Chris Esezobo	14th – Nurudeen Ikumapayi
3rd – Anietie Moses (1)	9th – Andreas Kresse	15th – Sharon Perry (2)
4th – Iruka Okeke (1)	10th – John Wain (2)	16th – Felixina Jonsyn-Ellis
5th – Laura Runyen-Janecky	11th – Joel Stavans	17th – Anietie Moses (2)
6th – Oladipo Aaron Aboderin	12th – Iruka Okeke (2)	

### **Titles/Abstracts of Presentations / Summaries of Participants**

Listed Alphabetically by Family Name [ Number and/or Type of Presentation(s) in Parentheses ]

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#### **Oladipo Aaron Aboderin [ Oral 6 ]**

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#### **PRELIMINARY REPORT ON ANTIBIOTIC RESISTANCE PROFILE OF *HELICOBACTER PYLORI* IN ILE-IFE, SOUTH-WEST, NIGERIA.**

By

**Aboderin A.O., Abdu, A-R, Odetoyin W, Okeke, I.N., Lawal O.O., Ndububa D A, Agbakwuru A.E. and Lamikanra A**

*Helicobacter pylori* has become recognized as a major cause of important gastroduodenal diseases in man. Evidence indicates that once acquired, *H. pylori* persists, usually for life unless eradicated by antimicrobial therapy. Over the past few years, we have accumulated some knowledge of the epidemiology of *H. pylori* in Ile-Ife, South-West Nigeria. In one collaborative study, we detected *H. pylori* in 195 (73%) patients referred for endoscopy at Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC). Furthermore we have observed a variegated gastric inflammatory response and atrophy including atrophic pangastritis but yet to demonstrate MALToma in a Nigerian patient. In addition we have demonstrated that dental plaque is a possible source of gastric *H. pylori* infection and such endogenous source could account for re-infection and difficulty in eradication

Presently, infected patients are treated with standard combination therapy and more recently, we sought to study antimicrobial susceptibility of locally isolated *H. pylori* strains.

We subjected 32 isolates to antimicrobial susceptibility testing against seven agents. All the isolates showed multiple resistance. All were resistant to amoxicillin, clarithromycin, metronidazole, while 29/31, 27/31 showed resistance to rifampicin and tetracycline respectively. Five (15.6%) of these isolates showed resistance to ciprofloxacin.

Our preliminary findings suggest that *H. pylori* strains tend to be more resistant to commonly used antibiotics and that strains exhibit multiresistance. On the basis of the findings it would be necessary to re-evaluate the eradication treatment regime in our setting.

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**Chris Esezobo [ Oral 8 ]**

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**KNOWLEDGE OF MEDICAL HOUSE-OFFICERS ABOUT ORAL REHYDRATION THERAPY AT LAGOS UNIVERSITY TEACHING HOSPITAL, LAGOS, NIGERIA.**

Esezobor CI. Department of paediatrics, Lagos University Teaching Hospital, Lagos, Nigeria.

**ABSTRACT**

**Introduction:** Oral rehydration therapy remains the mainstay of the management of most forms of dehydration associated with diarrhoea. However, studies in Nigeria and other developing countries have demonstrated improper use of oral rehydration solutions among care-givers. The author's interaction with care-givers confirms this inadequate knowledge. Improperly prepared oral rehydration solution constitutes a metabolic challenge to a child who is dehydrated and deaths have been reported after use of such solutions. Because doctors represent an important source of ORT knowledge to care-givers, this study aims to assess the quality of ORT knowledge among medical house-officers.

**Objectives :** To document the level of knowledge of pre-registration house-officers in Lagos University Teaching Hospital on the preparation and use of oral rehydration solutions in mild and moderate dehydration.

**Design:** A cross-sectional study of medical house-officers between November and December 2005. Relevant answers were obtained using questionnaires. Data were entered and analysed using EPI INFO 2002

**Result:** 80(68%) out of 120 medical house-officers returned their questionnaires. The male: female ratio was equal, about 69% of them were graduates of the University of Lagos medical school and 72.7% were in their first posting. Over a third were in paediatrics posting. 31.2% of the medical house-officers could correctly prepare salt-sugar solution while only about 25% of them would prescribe the correct volume of water to add to the sachet form of WHO-ORS; only a meagre 9 of the 77 respondents could correctly prepare both salt-sugar solution and WHO-ORS. About 37% and 27% of the doctors would give the right prescription for the correction of mild and moderate dehydration respectively. Graduation from medical school other than the University of Lagos college of medicine was associated with correctly preparing WHO-ORS, p value=0.007. Even after stratification by sex and present posting, it was still significant (p value=0.004). Present posting in paediatrics was associated with a significantly higher probability of properly correcting moderate dehydration, p value=0.038, but this did not reach statistical significance after stratification by sex and school of graduation. The male sex was positively associated with correct treatment of mild dehydration, p value=0.048. For moderate dehydration association with the male sex did not reach statistically significant level, p value=0.063

**Conclusion:** This study demonstrates low and inadequate knowledge of oral rehydration therapy among medical house-officers in the Lagos University Teaching hospital.

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**Deanna Gibson [ Oral 13 ]**

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***Salmonella* produces an O-Antigen capsule translocated by the *emc* operons, regulated by AgfD and important for environmental persistence.**

Gibson, D. L.,<sup>1</sup> White, A. P.,<sup>2</sup> Snyder, S. D.,<sup>3</sup> Heiss, C.,<sup>3</sup> Azadi, P.,<sup>3</sup>  
Surette M.,<sup>2</sup> and W. W. Kay<sup>1\*</sup>

Department of Biochemistry and Microbiology, University of Victoria, Victoria, V8W 3P6, B.C., Canada.<sup>1</sup> Department of Microbiology and Infectious Diseases, University of Calgary, Calgary, T2N 4N1, AB., Canada.<sup>2</sup> Complex Carbohydrate Research Center, Athens, 30602-4712, Georgia, USA.<sup>3</sup>

In this study, we show that *Salmonella* produces an O-Antigen capsule co-regulated with the extracellular matrix. Structural analysis of purified *Salmonella* extracellular polysaccharides yielded predominately a repeating oligosaccharide unit similar to that of *Salmonella* Enteritidis lipopolysaccharide O-Antigen with some modifications. Putative carbohydrate transport and regulatory operons important for capsule assembly and translocation, designated *emcA-H* and *emcI-J*, were identified by screening a random transposon library with immune serum generated to the capsule. The absence of capsule was confirmed by generating various in-frame  $\Delta emc$  mutants where *emcG* and *emcE* were shown to be important in capsule assembly and translocation. Additional *emc* genes may represent functional equivalents to group 1, group 4 and colanic acid capsule assembly and translocation components Wza, Wzb and Wzc, based on structural or functional similarities. Luciferase-based expression studies showed that, AgfD differentially regulated the *emc* operons in coordination with extracellular matrix genes coding for thin aggregative fimbriae and cellulose. Although the capsule did not appear to be important for multicellular behavior, we demonstrated it was important for survival during desiccation stress. The *emc* genes are conserved in *Salmonellae* and thus, the *Salmonella* extracellular matrix associated O-Antigen capsule may be a conserved survival strategy important for environmental persistence.

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**Usman Nurudeen Ikumapayi [ Oral 14 ]**

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**Title: Epidemiology and Molecular Characterization of Non-Typhoidal Salmonella Serovars in The Gambia**

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**Felixina Jonsyn-Ellis [ Oral 16 ]**

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**Untitled. A brief introduction to the University of The Gambia**

**Oral 9**

**Title: EU-Project EAC h Child: Combined Diagnostics for EPEC and Shigella**

**Poster**

**Title: A Rapid Immunological Detection Assay for Enteropathogenic *Escherichia coli* (EPEC)**

**Oral 3**

**In-vitro antimicrobial susceptibility studies of *E. coli* O157 and non-O157 serogroups isolated from animal and water sources in Northeastern Nigeria.**

1. \*Anietie E. Moses, Ph.D, AMLSCN, Department of Immunology, University of Maiduguri Teaching Hospital, PMB 1414, Maiduguri-Nigeria, E-mail: amoses264@yahoo.com

2. James A. Ameh, Ph.D, Department of Vet. Microbiology and Parasitology, University of Maiduguri, PMB 1069, Maiduguri-Nigeria. 3. Eno A. Moses, M. Sc., Department of Chemistry, University of Maiduguri, PMB 1069, Maiduguri-Nigeria. 4. Godwin O. Egwu, Ph.D (Professor of Vet. Medicine), Department of Vet. Medicine, University of Maiduguri, PMB 1069, Maiduguri-Nigeria, E-mail: egwug@hotmail.com

**Background:** Antibiotic resistance has been reported to be very high among *E. coli* isolates in developing countries. *E. coli* is recognized as a reservoir for resistance genes and can occupy multiple niches, including human and animal hosts. Routine monitoring of antibiotic resistance is therefore required to provide data for antibiotic therapy and resistance control.

**Method:** Strains of *E. coli* O157 and non-O157 serogroups isolated from cattle faeces, raw cow milk and water having significant phenotypic and pathogenic factors were studied for antimicrobial susceptibility using the agar disk diffusion, minimum inhibitory and minimum bactericidal concentrations to determine their susceptibility pattern.

**Results:** The results of agar disk diffusion test indicated that majority of the isolates from cattle and water exhibited high-level resistance to ampicillin, cephalixin, amoxicillin-clavulanic acid and moderately high resistance to tetracycline. These results correlated well with the high MIC values obtained in this study for ampicillin and tetracycline (12.5 - >50 µg/ml). Ciprofloxacin had the lowest MIC ranging from 0.049 – 0.195 µg/ml for all the isolates, followed by gentamycin, chloramphenicol and sulphamethoxazole-trimethoprim with MIC ranges of 0.195 – 3.13 µg/ml, 0.78 – 25.0 µg/ml and 0.78 - >50 µg/ml respectively. Tylosin and lincomycin showed consistently high MICs ranging from 12.5 – 50 µg/ml and 25 – 50 µg/ml respectively against both *E. coli* O157 and non-O157 strains. The fluoroquinolones, streptomycin and to some extent nalidixic acid were highly active against all cattle *E. coli* O157 and non-O157 strains. Generally, all strains tested in this study indicated 100% susceptibility with ciprofloxacin, streptomycin, gentamycin and chloramphenicol. Multiple drug resistance was exhibited by all the *E. coli* strains. Each strain exhibited resistance to at least 2 (18.2%) to a maximum of 5 (45.5%) drugs. No significant difference ( $P > 0.05$ ) was observed in the MIC determination values using either 104 cfu/ml or 106 cfu/ml as inoculum. The MBC range for most of the organisms was between 0.001 – 0.5 µg/ml.

**Conclusion:** The susceptibility pattern of *E. coli* isolates suggest that indiscriminate use of antibiotics in food animals such as cattle could lead to increase in multiple drug-resistant bacteria as a result of selective pressures.

**Key words:** *E. coli* O157, non-O157 *E. coli*, antimicrobial susceptibility pattern

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## Oral 17

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### **Detection of *E. coli* O157 and O26 serogroups in human immunodeficiency virus-infected patients with clinical manifestation of diarrhoea in Maiduguri, Nigeria.**

1. \*Anietie E. Moses, Ph.D, AMLSCN, Department of Immunology, University of Maiduguri Teaching Hospital, PMB 1414, Maiduguri-Nigeria, E-mail: amoses264@yahoo.com

2. James A. Ameh, Ph.D, Department of Vet. Microbiology and Parasitology, University of Maiduguri, PMB 1069, Maiduguri-Nigeria. 3. Godwin O. Egwu, Ph.D. (Professor of Vet. Medicine), Department of Vet. Medicine, University of Maiduguri, PMB 1069, Maiduguri-Nigeria, E-mail: egwug@hotmail.com

**Background:** With the emergence of HIV, an entirely new spectrum of gastroenteritis caused by previously unrecognized organisms is increasingly observed to be apparent. The implication of *E. coli* O157 and non-O157 serogroups, and their antibiotic resistance pattern in HIV-infected patients with diarrhoea in Maiduguri, Nigeria was studied.

**Methods:** One hundred and fifteen patients with symptoms of diarrhoea referred to Immunology Laboratory from the out-patient clinics of University of Maiduguri Teaching Hospital were screened for HIV-1 antibody. Stool samples were obtained and tested by microbiological methods.

**Results:** Of the 115 patients tested, 53 were HIV-positive of which 3(5.7%) had enteropathic *E. coli* serogroups in stool, comprising *E. coli* O157 (2; 3.8%) and O26 (1; 1.9%). None of the 59 HIV-seronegative control subjects tested harboured any of the enteropathic *E. coli* serogroups. The two *E. coli* O157 isolates were serologically detected using monospecific O157 antisera and O157 latex agglutination tests, and produced verotoxin-1 (VT1) as detected by Enzyme-Linked Immunosorbent Assay (sELISA). Vero cell culture of the strains and multiplex Polymerase Chain Reaction (mPCR) did not show cytopathic effect and virulence markers respectively. However, trimethoprim-sulphamethoxazole and tetracycline resistance were observed among the isolates with minimum inhibitory concentrations greater than 50 ug/ml.

**Conclusion:** Some pathogenic *E. coli* serogroups could play a very important role as opportunistic infections of HIV-infected persons with gastroenteritis and should not be overlooked in the management of HIV-infected patients with episode of diarrhoea.

**Key words:** *E. coli* O157, *E. coli* O26, HIV infection, Antibiotic resistance

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**Iruka Okeke [ Oral 4 & 12 ]**

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## Oral 4

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**Title: Molecular Epidemiology of Enteroaggregative *Escherichia coli***

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## Oral 12

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**Multiple antimicrobial resistance genes are carried by a second large plasmid in enteropathogenic *Escherichia coli* phylogenetic group EPEC2 strains**

Iruka N Okeke  
Department of Biology, Haverford College

Enteropathogenic *Escherichia coli* are an important cause of infantile diarrhea in developing countries. Typical EPEC strains possess a chromosomal island, the locus of enterocyte effacement, and a virulence plasmid, which carries genes encoding bundle forming pili and a plasmid encoded regulator. Two phylogenetic lineages of typical EPEC have been described with EPEC1 strains historically showing a greater association with outbreaks than EPEC2 strains. EPEC2 strains lack an *espC* island present in the EPEC1 group and a significant minority of them have lost the ability to make bundle-forming pili and the plasmid-encoded regulator, although they still carry the virulence plasmid. We performed subtractive hybridization using large plasmid preparations from one such strain as tester and

the plasmid from prototypical EPEC1 strain E2348/69 as driver. We isolated seven tester-specific fragments, five of which mapped to a conjugative system previously described in *Salmonella*. Further experiments demonstrated that the conjugative system is on a separate plasmid, which also carries multiple antimicrobial resistance genes. The second plasmid is present in most EPEC2 strains, including prototypical strains from this lineage, irrespective of whether they functional bundle-forming pili and plasmid-encoded regulator genes. The second plasmid appears to have been overlooked because it co-migrates with the virulence plasmid on standard gels. The presence of this multiresistance plasmid could account for the continued epidemiological relevance of EPEC2 strains in the face of antimicrobial pressure worldwide.

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**Sharon Perry [ Oral 2 & 15 & Poster x 2 ]**

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**Oral 2**  
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**Title: *Helicobacter pylori* and Risk of Secondary Gastroenteritis**

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**Oral 15**  
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**Title: Association of new *H. pylori* infection with exposure to gastroenteritis in *H. pylori*-infected persons**

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**Posters**  
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**Titles:**

**A - *H. pylori* and Risk of Gastroenteritis &**

**B - New *H. pylori* Infection is Associated with Exposure to Gastroenteritis in *H. pylori*-infected Household Contacts**

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**Laura Runyen-Janecky [ Oral 5 ]**

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The Inside Story: *Shigella flexneri*'s life in the eukaryotic cell  
Laura Runyen-Janecky  
University of Richmond, USA

The facultative intracellular bacterium *Shigella flexneri* causes bacterial dysentery in humans as a result of its multiplication within colonic epithelial cells. Exposure to this eukaryotic intracellular environment induces the expression of a particular set of bacterial genes, some of which facilitate intracellular growth and survival. To identify genes *S. flexneri* that are induced in response to the colonic epithelial intracellular environment, we screened a library containing fragments of the *S. flexneri* chromosome fused to a promoterless green fluorescent protein gene (*gfp*). Bacteria containing promoter fusions that had a higher level of *gfp* expression when *S. flexneri* were intracellular (in Henle cells) than when *S. flexneri* were extracellular (in LB broth) were isolated using fluorescence activated cell sorting. We isolated genes encoding proteins that are involved in a wide variety of processes including metabolism, phosphate uptake/regulation, and high affinity divalent metal uptake/metabolism. These results have been verified and expanded in microarray studies from other labs. Many, but not all, of the genes that are induced when *Shigella* is within the eukaryotic cell are also induced when other pathogens such as *Salmonella* and *Listeria* are intracellular. To determine whether the *Shigella* intracellular induced genes are required for survival or growth in the intracellular environment, we constructed mutations in several of these induced genes. The *Shigella uhpT* mutant, which is defective in utilization of glucose-6-phosphate as a sole carbon source *in vitro*, exhibited normal plaque formation on Henle cell monolayers, indicating that the bacterium could multiply in the host cell cytoplasm. Strains carrying single mutations in the iron or manganese transporters formed wild type plaques on Henle cell monolayers, indicating *Shigella* was able to acquire iron and/or manganese in the host cell. However, double mutants in two iron acquisition systems formed slightly smaller plaques on Henle cell monolayers, and a *Shigella* mutant defective in three systems did not form plaques. A *Shigella* strain carrying mutations in both of the known manganese transport systems formed wild type plaques on Henle cell monolayers, but had a reduced ability to survive in activated macrophages. In sum, numerous genes are specifically induced when *Shigella* is intracellular and encode proteins that contribute to

intracellular growth and survival of *Shigella*. Many of these induced genes also contribute to the intracellular growth and survival of other intracellular pathogens.

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**Joel Stavans [ Oral 11 ]**

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**Title: Induction of the Lambda Phage**

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**John Wain [ Oral 1 & 10 ]**

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**Oral 1**  
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**Title: Typing Salmonella; Molecular Options**

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**Oral 10**  
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**Title: The Problem with Resistance in Enteric Fever Pathogens**

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**Aaron White [ Oral 7 ]**

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*Salmonella* Multicellular Behavior and Environmental Persistence

*Salmonella* spp. are environmentally persistent pathogens that have served as important models for understanding how bacteria adapt to stressful conditions. However, it remains poorly understood how they survive extreme conditions encountered outside their hosts. Many isolates form dry and rough (rdar) colonies with elaborate surface patterns when grown for several days on standard laboratory agar media. This phenotype has been characterized as a multicellular behavior and termed the rdar morphotype. Cells in rdar colonies are encased in an aggregative extracellular matrix comprised of several polymeric substances: thin aggregative fimbriae (Tafi; curli), cellulose and at least two novel extracellular polysaccharides. We tested the prevalence of the rdar morphotype in diverse *Salmonella* isolates that have been grouped into Reference collection B (SARB; 72 strains, subgroup I, human isolates) and Reference collection C (SARC; 96 strains, subgroups I-VII, environmental isolates). Rdar prevalence was high in both collections and neared 85-90%. Furthermore, by studying luciferase transcriptional fusions to key promoter sequences we confirmed that production of the extracellular matrix components in *Salmonella* has been functionally conserved, despite divergence in promoter sequences. This is significant because rdar morphotype cells have enhanced resistance to desiccation and commonly used disinfectants (i.e., sodium hypochlorite) compared to mutants deficient in Tafi and/or cellulose production. Aggregation of cells in the rdar morphotype may ensure sufficient numbers for survival outside the host. In addition, the presence of hardy substances like fimbriae, cellulose and EPS may aid in passage of *Salmonella* through the mammalian stomach thereby reducing the infectious dose.

Conference Bio

Dr. Aaron P. White is a Research Associate with Dr. Michael G. Surette in the Department of Microbiology and Infectious Diseases at the University of Calgary and completed his Ph.D. in Biochemistry (2000) with Dr. William W. Kay at the University of Victoria, CANADA.

For the past five years, I have been studying the role of the extracellular matrix in the long-term survival of *S. enterica* serovars Enteritidis and Typhimurium. Research with Dr. Surette has focused on the use of luciferase-based promoter fusions to determine the genetic conservation of factors involved in extracellular matrix production throughout the entire *Salmonella* genus. Work in collaboration with Dr. Kay and Dr. Deanna Gibson (also presenting at this meeting) has focused on biochemical characterization of different components that comprise the extracellular matrix. Understanding *Salmonella* survival in non-host environments will help to develop more effective control measures to reduce future disease outbreaks.