

Arsenic metabolizing microbes in urogenital schistosomiasis and induced bladder pathologies in eggua, Nigeria

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Abstract

Research into host microbes and their metabolites are important as they may be capable of influencing host health and disease. Recent evidence indicates arsenic metabolic processes may have roles in carcinogenesis. Therefore, the aim of this study was to determine the presence, abundance and potentials of arsenic metabolizing microbial genes and microbes in urogenital schistosomiasis and induced bladder pathologies. Previously, microbiome data was obtained using 16S next-generation sequencing from urine samples of individuals from Eggua in South western Nigeria and categorized with respect to schistosomiasis and bladder pathologies. Bioinformatics analytical tools were used to determine presence and to identify arsenic metabolizing genes from the sequence data. *Staphylococcus aureus* was the most abundant in the advanced category, and *Staphylococcus sciuri* was the most abundant in the control category. Arsenic metabolizing genes were present in some species distributed among all the categories in different levels. In some of the species, the most common arsenic associated gene was arsenate reductase gene (*arsC*). The study improves knowledge of potential mechanisms involved in schistosomiasis-induced cancer.

Keywords: Arsenic, Bioinformatics, Bladder Pathology, Metabolism, Microbes, Schistosomiasis

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I. Introduction

The microbiome in man is composed of bacteria, archaea, viruses and fungi, which are predominantly found in the gastrointestinal tract, but also in other exposed tissues, such as the skin, upper respiratory and urogenital tracts (Ursell, et al., 2012; Whiteside, et al., 2015). There is microbiome associated with the healthy urinary tract that can change in urologic disorders (Aragon et al., 2016). In the study carried out by Adebayo et al., (2017) in Eggua, persons infected with the parasite *Schistosoma haematobium* or had developed bladder complications along with the parasite infection, shared a large portion of organisms in their urinary tract microbiome, and there was a microbe's genus unique to infected persons and those with bladder problems. Microbes do not necessarily cause disease such as cancer, but their presence or absence in different sites of the human body usually influence body functions (Adebayo, et al., 2017), and this is mostly due to their biological activities, such as metabolism (Stolz, et al., 2010). Products of bacterial metabolism are believed to modulate human health (Hooper, et al., 2012) in many ways both positively and negatively (Sharon et al., 2014). Over 25,000 microbial metabolites have been reported in the scientific literature and some of the toxic metabolites that affect or contribute to bladder cancer formation and progression have been suggested to come from the host's resident microbes (Bioaustralis, 2018). The effect of metabolites differs depending on the metabolite and site in the body of the host (Postler and Ghosh 2017) and they can promote tumorigenesis, even at distant body sites (Popovic et al., 2018). The host and bacterial polyamine metabolites have been suggested to synergistically promote biofilm formation and cancer growth, creating conducive conditions for the transformation of normal cells to cancer cells (Johnson et al., 2015).

Arsenic has been found to be present in food, water and air. The influence of arsenic on the composition and function of the human-associated microbiota cannot be overemphasized in human health and disease (Isokpehi, et al., 2014). Low doses and long-term exposures to arsenic lead to a range of medical complications termed "Arsenicosis" (Bakare, et al., 2018). The severity and the adverse effect of arsenic on

human health depend on its metabolites (De Chaudhuri, et al., 2008). Studies have shown that monomethylarsenic acid (MMA), though being a methylated metabolite, is the most toxic Arsenic metabolite (De Loma, et al., 2018).

To the scope of this research, arsenic metabolizing microbes were identified to know if their ability to metabolize arsenic influences their abundance in individual with schistosomiasis and bladder pathology.

II. Methods

2.1 Data set

We made use of the sequences previously obtained from 70 urine samples that had been categorized into four: Advanced, Pathology only, Infection only, and Control (Adebayo, et al., 2017). Data and project information were retrieved from NCBI's Sequence Read Archives, SRA, under accession SRP094688. The pathology only category had total number of 9 samples and so from each of the other categories, 9 randomly selected representative samples were taken, making the total number of 36 urine samples used for this study.

2.2 Identification of species

Species of microbes present in each sample were identified using NCBI BLAST. The unknown representative sequences were copied to NCBI (<http://blast.ncbi.nlm.nih.gov/>) for BLAST search. Under Basic BLAST, Nucleotide BLAST (blastn) was selected and representative sequences were pasted in the query box. Adjusted parameters include selection of 16S ribosomal RNA sequences (Bacterial and Archaea) for database search and highly similar sequences (megablast) under program selection. These two adjustments were maintained throughout the BLAST search for all queries. The most rated of all the hits was selected as the specie for each query sequence, considering the hit with E-value of 0.0, Percentage Identity of 70 and above, and highest Query Cover. Were the above conditions were not met, such BLAST results were not considered.

2.3. Abundance of identified species

On Microsoft Excel 2016, the relative abundance of each identified species at category level was calculated from the previously generated sequence abundance data for each sample by Adebayo *et al.* (2017).

2.4. Availability of arsenic metabolizing in identified species

The complete genomes of identified species in each category were searched for on Genome Online Database (<https://gold.jgi.doe.gov/>) and NCBI Nucleotide database (<https://www.ncbi.nlm.nih.gov/nucleotide/>). Species that have its complete genome (available in strain(s)) already deposited in these databases were downloaded in GenBank(full) format. Each microbial genome downloaded was integrated into a visual analytic software Artemis 18.0.2 (<http://sanger-pathogens.github.io/Artemis/Artemis/>). In each successfully loaded genome on Artemis, the presence of the well characterized arsenic-associated genes: :anion-transporting ATPase (ArsA), arsenical pump membrane protein (ArsB), arsenate reductase (ArsC), arsenical resistance operontransacting repressor (ArsD), andAs(III)-responsive transcriptionalrepressor (ArsR) gene for arsenic metabolism (Isokpehi, et al., 2014) were searched for.

III. Results

3.1. Identified species

During this research, 1432 species found in the database were identified. These identified species belong to 496 genera including *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Vibrio*, *Corynebacterium*, *Lactobacillus*, *Paenibacillus*, *Streptococcus*, *Chryseobacterium* and others. All the identified species were bacteria distributed across 13 Phyla. Phylum Proteobacteria and Phylum Firmicutes had the highest occurrence. Others include Actinobacteria, Bacteroidetes, Cyanobacteria, Deinococcus, Fusobacteria, Chloroflexi, Tenericutes, Acidobacteria, Balneolaeota, Elusimicrobia, Gemmatimonadetes. Some of the species identified include: *Staphylococcus aureus*, *Pseudomonas parafulva*, *Acinetobacter junii*, *Staphylococcus sciuri*, *Pseudomonas plecoglossicida*, *Pseudomonas gessardii*, *Acinetobacter seohaensis*, *Moraxella osloensis*, *Acinetobacter junii*, etc.

3.2. Abundance of species

Abundance of identified species varies from one category to another. From the Advanced category (Figure 1a), 580 bacteria species were identified. *Staphylococcus aureus* had the highest relative abundance of 11.3%, followed by *Acinetobacter junii* that had 10.2%, *Pseudomonas parafulva* had 10.0%, *Staphylococcus sciuri* had 9.7%. The above-mentioned species were obviously more abundant in the category as revealed in the figure. In pathology only category (Figure 1b), 716 bacteria species were identified. *Pseudomonas parafulva* had the highest relative abundance of 17.5% and it was followed by *Pseudomonas plecoglossicida* that had 5.5% relative abundance. *P. parafulva* is far more abundant than *P. plecoglossicida* by 12.0%. It is evident therefore that *Pseudomonas parafulva* highly dominated the category. From the Infection only category (Figure

1c), 647 bacteria species were identified. *Pseudomonas parafulva* had 15.9% as the highest relative abundance and followed by *Staphylococcus saprophyticus* that had 11.5% relative abundance. The figure shows that some species aside *Pseudomonas parafulva* were abundant in the category and most occurring genus in the category is *Pseudomonas*. In Control category (Figure 1d), 843 species were detected. The highest relative abundance of 31.2% belongs to *Staphylococcus scuri* and followed by *Acinetobacter haemolyticus* that had 16.2%. *Staphylococcus scuri* was more abundant than *Acinetobacter haemolyticus* with not less than 15.0%.

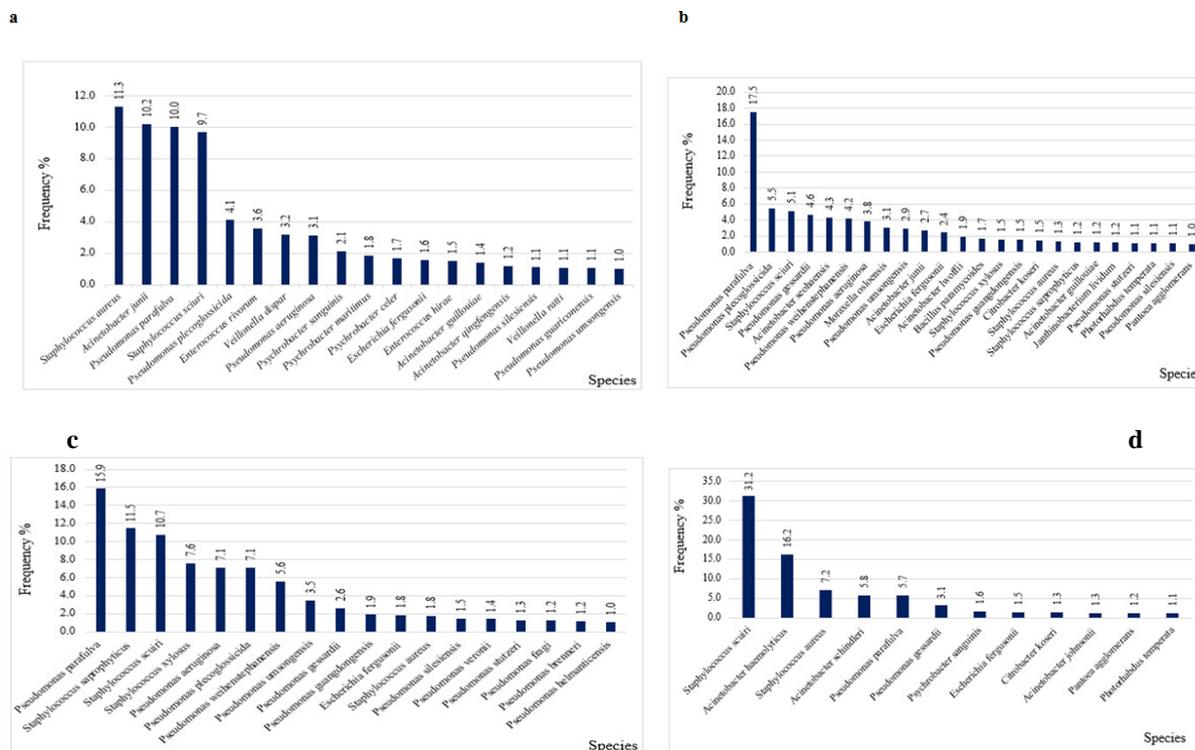


Figure 1. Identified species that had relative abundance $\geq 1\%$ in each category. (See legend on next page)

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Figure 1. Identified species that had relative abundance $\geq 1\%$ in each category

a. In Advanced category, *Staphylococcus aureus* had the highest relative abundance of 11.3%, followed by *Acinetobacter junii* that had 10.2%, *Pseudomonas parafulva* had 10.0%, *Staphylococcus scuri* had 9.7%.

b. Pathology only category. *Pseudomonas parafulva* is far more abundant than *P. plecoglossicida* by 12.0%.

c. Infection only category. Most occurring genus in the category is *Pseudomonas*.

d. Control category. *Staphylococcus scuri* was more abundant than *Acinetobacter haemolyticus* with not less than 15.0%.

Note: Other identified species not shown in the figure had less than 1% relative abundance.

3.3. Presence of arsenic metabolizing genes in identified species

Identified species complete genome available in Genome Online Database (GOLD) and NCBI Nucleotide database during the period of this research were downloaded. Out of those that have their complete genome in the databases, only the species annotated to have genes of interest and had relative abundance $\geq 1\%$ were reported. Most of the species complete genome available in the databases was available as strains. Therefore, some of the available strains for each species were compared.

3.3.1. Arsenic metabolizing genes in advanced category

Figure 2(a) shows Arsenic-associated genes in strains of identified species that had relative abundance $\geq 1\%$ in the Advanced category. Arsenic-associated genes were identified in two strains of *Staphylococcus aureus* (MRSA252 and IT1-S). Strain MRSA252 had three arsenic-associated genes (*arsB*, *arsC* and *arsR*) but only *arsC* gene was identified in strain IT1-S. Two strains of *Acinetobacter junii* (Izh-X15 and WCHAJ59) had three arsenic-associated genes. *arsB* gene and *arsC* gene were identified in both strains, but strain Izh-X15 had *arsR* gene while strain WCHAJ59 had *arsH* gene. In *Pseudomonas parafulva*, arsenic-associated genes were identified in two strains (JBCS1880 and CRS01-1). Strain JBCS1880 had *arsB* and *arsC* genes while in strain

CRS01-1, only *arsC* gene was identified. Arsenic-associated genes were in two strains of *Pseudomonas aeruginosa* (PA96 and HS9). Strain PA96 had two genes (*arsB* and *arsC*) while strain HS9 had three genes (*arsB*, *arsC* and *arsH*). In the genome of *Acinetobacter guillouiae* NBRC 110550, three arsenic-associated genes (*arsB*, *arsC*, and *arsH*) were present. In the strains of other species such as *Staphylococcus sciuri*, *Pseudomonas plecoglossicida*, *Escherichia fergusonii* and *Enterococcus hirae*, only *arsC* genes was identified.

a. Advance Only

Genome	arsA	arsB	arsC	arsD	arsH	arsR
<i>Pseudomonas parafulya</i> strain JBCS1880		■	■			
<i>Pseudomonas parafulya</i> strain CRS01-1			■			
<i>Pseudomonas plecoglossicida</i> XSDHY-P			■			
<i>Staphylococcus sciuri</i> FDAARGOS 285			■			
<i>Pseudomonas aeruginosa</i> PA96		■	■			
<i>Pseudomonas aeruginosa</i> HS9		■	■		■	
<i>Moraxella osloensis</i> KSH						
<i>Acinetobacter junii</i> strain lzh-X15		■	■			■
<i>Acinetobacter junii</i> strain WCHAJ59		■	■			
<i>Escherichia fergusonii</i> 40A			■			
<i>Acinetobacter iwoffii</i> ZS207		■	■		■	
<i>Citrobacter koseri</i> AR 0024						
<i>Citrobacter koseri</i> FDAARGOS 287			■			
<i>Staphylococcus aureus</i> strain IT1-S						
<i>Staphylococcus aureus</i> strain MRSA252		■	■			■
<i>Staphylococcus saprophyticus</i> FDAARGOS 137						
<i>Staphylococcus saprophyticus</i> FDAARGOS 336						
<i>Acinetobacter guillouiae</i> NBRC 110550		■	■		■	
<i>Pseudomonas stutzeri</i> SGAir0442		■	■		■	
<i>Pseudomonas stutzeri</i> FDAARGOS 355		■	■		■	
<i>Pseudomonas stutzeri</i> DW2-1		■	■		■	
<i>Pantoea agglomerans</i> CFSAN047153			■			

b. Pathology Only

Genome	arsA	arsB	arsC	arsD	arsH	arsR
<i>Staphylococcus aureus</i> strain IT1-S			■			
<i>Staphylococcus aureus</i> strain MRSA252		■	■			■
<i>Acinetobacter junii</i> strain lzh-X15		■	■			■
<i>Acinetobacter junii</i> strain WCHAJ59		■	■		■	
<i>Pseudomonas parafulya</i> strain JBCS1880		■	■			
<i>Pseudomonas parafulya</i> strain CRS01-1			■			
<i>Staphylococcus sciuri</i> FDAARGOS 285			■			
<i>Pseudomonas plecoglossicida</i> XSDHY-P			■			
<i>Pseudomonas aeruginosa</i> PA96		■	■			
<i>Pseudomonas aeruginosa</i> HS9		■	■		■	
<i>Escherichia fergusonii</i> 40A			■			
<i>Enterococcus hirae</i> FDAARGOS 234			■			
<i>Acinetobacter guillouiae</i> NBRC 110550		■	■		■	

c. Infection Only

Genome	arsA	arsB	arsC	arsD	arsH	arsR
<i>Pseudomonas parafulva</i> strain JBCS1880		■	■			
<i>Pseudomonas parafulva</i> strain CRS01-1			■			
<i>Staphylococcus saprophyticus</i> FDAARGOS 137			■			
<i>Staphylococcus saprophyticus</i> FDAARGOS 336			■			
<i>Staphylococcus sciuri</i> FDAARGOS 285			■			
<i>Pseudomonas aeruginosa</i> PA96		■	■			
<i>Pseudomonas aeruginosa</i> HS9		■	■		■	
<i>Pseudomonas plecoglossicida</i> XSDHY-P			■			
<i>Pseudomonas gessardii</i> BS2982			■		■	
<i>Escherichia fergusonii</i> 40A			■			
<i>Staphylococcus aureus</i> strain IT1-S			■			
<i>Staphylococcus aureus</i> strain MRSA252		■	■			■
<i>Pseudomonas stutzeri</i> SGAir0442		■	■			
<i>Pseudomonas stutzeri</i> FDAARGOS 355		■	■			
<i>Pseudomonas stutzeri</i> DW2-1		■	■			
<i>Pseudomonas fragi</i> DBC			■		■	

d. Control Group

Genome	arsA	arsB	arsC	arsD	arsH	arsR
<i>Staphylococcus sciuri</i> FDAARGOS 285			■			
<i>Acinetobacter haemolyticus</i> sz1652		■	■		■	
<i>Staphylococcus aureus</i> strain IT1-S			■			
<i>Staphylococcus aureus</i> strain MRSA252		■	■			■
<i>Acinetobacter schindleri</i> ACE			■		■	
<i>Pseudomonas parafulva</i> strain JBCS1880		■	■			
<i>Pseudomonas parafulva</i> strain CRS01-1			■			
<i>Pseudomonas gessardii</i> BS2982			■		■	
<i>Escherichia fergusonii</i> 40A			■			
<i>Citrobacter koseri</i> AR 0024			■			
<i>Citrobacter koseri</i> FDAARGOS 287			■			
<i>Acinetobacter johnsonii</i> IC001		■	■		■	
<i>Acinetobacter johnsonii</i> LXL C1		■	■		■	
<i>Acinetobacter johnsonii</i> M19		■	■		■	
<i>Pantoea agglomerans</i> CFSAN047153			■			

Figure 2. (a) Arsenic metabolizing genes in the strains of identified species that had relative abundance \geq 1%. Note: Black color for presence of gene.

3.3.2. Arsenic metabolizing genes in pathology only category

Figure 2(b) shows arsenic-associated genes in strains of identified species that had relative abundance \geq 1% in the Pathology only category. In *Pseudomonas parafulva*, arsenic-associated genes were identified in two strains (JBCS1880 and CRS01-1). Strain JBCS1880 had *arsB* and *arsC* genes while in strain CRS01-1, only *arsC* gene was identified. Arsenic-associated genes were identified in two strains of *Pseudomonas aeruginosa* (PA96 and HS9). Strain PA96 had two genes (*arsB* and *arsC*) while strain HS9 had three genes (*arsB*, *arsC* and *arsH*). Two strains of *Acinetobacter junii* (Izh-X15 and WCHAJ59) had three arsenic-associated genes. *arsB* gene and *arsC* gene were identified in both strains, but strain Izh-X15 had *arsR* gene while strain WCHAJ59 had *arsH* gene. Three arsenic-associated genes (*arsB*, *arsC* and *arsH*) were identified to be present in *Acinetobacter iwoffi* ZS207. Arsenic-associated genes were identified in two strains of *Staphylococcus aureus* (MRSA252 and IT1-S). Strain MRSA252 had three arsenic-associated genes (*arsB*, *arsC* and *arsR*) but only *arsC* gene was identified in strain IT1-S. In the genome of *Acinetobacter guillouiae* NBRC 110550, three arsenic-

associated genes (*arsB*, *arsC*, and *arsH*) were present. All the strains of *Pseudomonas stutzeri* in the figure had three arsenic associated genes (*arsB*, *arsC* and *arsH*). *Pseudomonas plecoglossicida*, *Staphylococcus sciuri*, *Moraxella osloensis*, *Escherichia fergusonii*, *Citrobacter koseri*, *Staphylococcus saprophyticus* and *Pantoea agglomerans* only had *arsC* gene.

3.3.3. Arsenic metabolizing genes in infection only category

Figure 2(c) shows arsenic-associated genes in strains of identified species that had relative abundance greater than or equal to 1% in the Infection only category. In *Pseudomonas parafulva*, arsenic-associated genes were identified in two strains (*JBCS1880* and *CRS01-1*). Strain *JBCS1880* had *arsB* and *arsC* genes while in strain *CRS01-1*, only *arsC* gene was identified. Arsenic-associated genes were identified in two strains of *Pseudomonas aeruginosa* (*PA96* and *HS9*). Strain *PA96* had two genes (*arsB* and *arsC*) while strain *HS9* had three genes (*arsB*, *arsC* and *arsH*). The only one strain of *Pseudomonas gessardii* (*BS2982*) reported in the figure had two arsenic-associated genes (*arsC* and *arsH*). Arsenic-associated genes were identified in two strains of *Staphylococcus aureus* (*MRSA252* and *IT1-S*). Strain *MRSA252* had three arsenic-associated genes (*arsB*, *arsC* and *arsR*) but only *arsC* gene was identified in strain *IT1-S*. Three strains of *Pseudomonas stutzeri* (*SGAir0442*, *FDAARGOS_355* and *DW2-1*) had the same three arsenic associated genes (*arsB*, *arsC* and *arsH*). *Pseudomonas fragistra* had *arsC* and *arsH* genes. In *Staphylococcus saprophyticus*, *Staphylococcus sciuri*, *Pseudomonas plecoglossicida*, and *Escherichia fergusonii*, only *arsC* genes was identified.

3.3.4. Arsenic metabolizing genes in control category

Figure 2(d) shows arsenic-associated genes in strains of identified species that had relative abundance \geq 1% in the Control only category. In *Acinetobacter haemolyticus sz1652*, three arsenic-associated genes (*arsB*, *arsC* and *arsH*) were identified. Arsenic-associated genes were identified in two strains of *Staphylococcus aureus* (*MRSA252* and *IT1-S*). Strain *MRSA252* had three arsenic-associated genes (*arsB*, *arsC* and *arsR*) but only *arsC* gene was identified in strain *IT1-S*. In *Acinetobacter schindleri ACE*, three genes (*arsB*, *arsC*, and *arsH*) were identified. In *Pseudomonas parafulva*, arsenic-associated genes were identified in two strains (*JBCS1880* and *CRS01-1*). Strain *JBCS1880* had *arsB* and *arsC* genes while in strain *CRS01-1*, only *arsC* gene was identified. The strain of *Pseudomonas gessardii* (*BS2982*) reported in the figure had two arsenic-associated genes (*arsC* and *arsH*). The same arsenic-associated genes (*arsB*, *arsC* and *arsH*) were identified in three strains of *Acinetobacter johnsonii*. *Staphylococcus sciuri*, *Escherichia fergusonii*, *Citrobacter koseri* and *Pantoea agglomerans* only had *arsC* genes.

3.3.5. Arsenic metabolizing genes in some less abundant species in all categories

In figure 3, some identified species that were less abundant in their respective categories had arsenic-associated genes. Also, in the genome of some e.g., *Staphylococcus xylosum* (strain S170, SMQ-121 and HKUOPL8), arsenic-associated gene was not found.

Genome	arsA	arsB	arsC	arsD	arsH	arsR
<i>Acinetobacter baumannii</i> ABNIH28						
<i>Escherichia fergusonii</i> 40A						
<i>Citrobacter koseri</i> AR_0025						
<i>Acinetobacter dyoffi</i> ZS207						
<i>Staphylococcus aureus</i> MRSA252						
<i>Pseudomonas aeruginosa</i> PA96						
<i>Pseudomonas aeruginosa</i> HS9						
<i>Pseudomonas plecoglossicida</i> XSDHY-P						
<i>Pseudomonas stutzeri</i> SGAir0442						
<i>Escherichia coli</i> MS 198-1						
<i>Acinetobacter johnsonii</i> IC001						
<i>Acinetobacter schindleri</i> ACE						
<i>Pseudomonas gessardii</i> BS2982						
<i>Staphylococcus epidermidis</i> SK135						
<i>Staphylococcus epidermidis</i> FDAARGOS_529						
<i>Moraxella osloensis</i> KMC41						
<i>Staphylococcus hominis</i> FDAARGOS_575						
<i>Escherichia coli</i> MS 115-1						
<i>Staphylococcus saprophyticus</i> FDAARGOS_137						
<i>Acinetobacter junii</i> strain 1zh-X15						
<i>Acinetobacter haemolyticus</i> strain sz1652						

Figure 3. Presence of arsenic metabolizing genes in strains of some identified less abundant species across all category.

3.3.6. Arsenic metabolizing genes in most abundant species from each category

In the Advanced category, complete genome of three strains for *Staphylococcus aureus* were checked. Only strain *Newman_D2C* had three genes (*arsB*, *arsC* and *arsR*). In pathology category and infection category, *Pseudomonas parafulva* was the most abundant and strain *JBCS1880* had *arsB* and *arsC*. *Staphylococcus sciuri* was the most abundant in the control category and in strain *FDAARGOS_285* representing the species, only *arsC* gene was present.

3.4 Discussion

In this study, we identified arsenic metabolizing microbes present in 36 urine samples from previously collected 70 urine samples from volunteers in Eggua, Yewa North Local Government Area in Ogun State, Southwestern Nigeria. We also compared most abundant species to less abundant species to know if actually, the ability to metabolize arsenic influences their abundance in each category. In agreement with earlier report (Adebayo et al., 2017), in this study, the sequence abundance data shows that abundant species, such as *Staphylococcus aureus* and *Pseudomonas parafulva* belongs to two phyla: Firmicutes and Proteobacteria and other species belongs to other phyla such as Actinobacteria, Fusobacteria, Bacteroidetes, Gemmatimonadetes, etc.

Although, the relative abundance of the identified species in each category revealed that *Staphylococcus aureus* was the most abundant species in the Advance category (Figure 1a), the dominating species in Pathology only category and Infection only category were also abundant in the advanced category, been the category with the two health conditions (infection and pathology). The figure shows that *Staphylococcus aureus*, *Acinetobacter junii*, *Pseudomonas parafulva*, and *Staphylococcus sciuri* were relatively close in their relative abundance: 11.34%, 10.20%, 10.01%, and 9.69% respectively. Reports already confirmed that all the first four most abundant species in the Advanced category belongs to genera that have been implicated to enhance infection such as UTIs and initiate or promote pathogenesis (Wu, et al., 2018; Salavati, et al., 2018). Both Pathology only category and Infection only category had *Pseudomonas parafulva* as their most abundant species (Figure 1b; Figure 1c), but the species dominated Pathology only category than Infection only category. In Pathology only category, *Pseudomonas parafulva* relative abundance was 17.5% compared to the second most abundant of 5.5% and this shows that *Pseudomonas parafulva* indeed dominated the Pathology only category (Figure 1b). In the Infection only group, although *Pseudomonas parafulva* was the most abundant species with 15.9% relative abundance, and the second most abundant species was *Staphylococcus saprophyticus* with 11.5% relative abundance. *Staphylococcus sciuri* is the most abundant species in control category (Figure 1d).

Most of the species identified were found in all the categories, such as *Staphylococcus epidermidis* and *Escherichia coli*. Also, a few of the identified species were found only in one category e.g., *Fusobacterium gastrois*, a possible pro tumorigenic pathogen (Popovic, et al., 2018) was found in the Advance category, *Massilia aurea* in pathology only category, *Virgibacillus proomii* in infection category and *Psychrobacter pacificensis* in the control category. In the infection only category, the genus *Pseudomonas* was dominant. Oluyombo, et al., (2019) reported that the genus *Pseudomonas* through a mechanism yet unknown, easily dominate during infection and *Pseudomonas aeruginosa* for example has been found to be on the rise in Urinary Tract Infection (Shah et al., 2015).

Evidence confirms that the microorganisms inhabiting many sites of the body produce metabolites that influence health status (Hughes and Rowland, 2000) including the urinary tract microbes (Whiteside, et al., 2015). Some microbial metabolites can promote infection and are capable of inducing inflammation, initiate or promote carcinogenesis (Stone et al., 2016). Arsenic, a type 1 carcinogen, influences the composition and function of the human-associated microbiota and this is of significance in human health and disease (Isokpehi, et al., 2014). The severity and adverse effects of Arsenic on human health depends on its metabolism (De Chaudhuri, et al., 2008).

In this study, the genomes of the available strains of the most abundant species in each category of volunteers were searched for the presence of arsenic metabolizing genes. *Staphylococcus aureus* MRSA252 in the Advanced category had arsenic metabolizing genes (*arsB*, *arsC*, and *arsR*) in its genome (Figure 2a), *Pseudomonas parafulva* JBCS1880 in Pathology only and Infection only have two of the arsenic-associated genes (*arsB* and *arsC*) in its genome (Figure 2b and 2c) and only one arsenic associated gene (*arsC*) was found to be present in the strain representing the most abundant species in the control category i.e., *Staphylococcus sciuri* FDAARGOS_285 (Figure 2d). By number, arsenic-associated genes were more present in the most abundant species of the advance category compared to the most abundant species in other categories.

However, the presence of arsenic-associated genes in some less abundant detected species across the categories showed that, most of these species have in their genome, a good number of arsenic associated genes. For example, *Staphylococcus epidermidis* was identified to be present in all the categories but its relative abundance in each category was less than 1% and all the available strains of *Staphylococcus epidermidis* shows

high number of arsenic-associated genes in their genomes (Isokpehiet al., 2014), e.g., *Staphylococcus epidermidis* SK135 had good number of these genes: *arsA*, *arsB*, *arsC*, *arsD* and *arsR* (Figure 3). Also, *Escherichia coli*, known to be responsible for 70% of urinary infection and may also play a major additive and synergistic role during bladder carcinogenesis (El-Mosalamy, et al., 2012) was identified in all categories but its relative abundance was less than 1% in each category. In its strain, *Escherichia coli* MS 198-1, *arsA*, *arsB*, *arsC*, *arsD*, *arsH* and *arsR* genes were found (Figure 3). In all the microbial genomes searched, the most common arsenic associated gene was *arsC*, and this is due to the fact that it is the main arsenic reductase. Also, *arsB*, the arsenical pump membrane gene was also common.

IV. Conclusion

Arsenic-associated genes were found to be present in the most abundant species across all categories. This indicated that, with appropriate regulators, these species have the ability to metabolize arsenic. Meanwhile, some less abundant species in all the four categories also have the arsenic-associated genes in their genomes. Some of these less abundant species can be said to have more ability to metabolize arsenic than the most abundant species, owing to the fact that they have more of these genes in their genome compared to the most abundant specie of each category. The information in this study reveals that, the abundance of these species in their respective categories was not necessarily influenced by their ability to metabolize arsenic, but more of a contributing factor for their survival. For future studies, it is recommended that the investigation should be carried out using whole genome sequences, which could lead to more accurate species identity and specific strain type in the urine samples. Also, aside Artemis 18.0.2 visual analytical software that was used for this study, two or more visual analytical software should be used and their gene search results should be compared.

References

- [1]. Adebayo, A.S., Survayanshi, M., Bhute, S., Agunloye, A.M., Isokpehi, R.D., Anumudu, C.I., Shouche, Y.S. (2017). The microbiome in urogenital schistosomiasis and induced bladder pathologies. *PLoS Neglected Tropical Diseases*, 11, 8.
- [2]. Bakare, S.O., Adebayo, A.S., Awobode, H.O., Onile, S.O., Agunloye, A.M., Isokpehi, R.D., Anumudu, C.I. (2018). Arsenicosis in bladder pathology and schistosomiasis in Eggua, Nigeria. *Trans R Soc Trop Med Hyg*, 1–8
- [3]. Bioaustralis (2018). *Microbial Metabolites*. Retrieved July 2018, from bioaustralis fine chemicals: <http://www.bioaustralis.com/metabolites.htm>
- [4]. De Chaudhuri, E., Ghosh, P., Sarma N., Majumdar P., Sau, T.J., Basu, S., Roychoudhury, S., Giri, A.K. (2008). Genetics Variants Associated with Arseni Susceptibility: Study of Purine Nucleoside Phosphorylase, Arsenic (13) Methyltransferase, and Glutathione S-Transferase Omega Genes. *Environ Health Perspect*, 116, 501-505.
- [5]. De Loma, J., Skróder, H., Raqib, R., Vahter, M., Broberg, K. (2018). Arsenite methyltransferase (AS3MT) polymorphisms and arsenic methylation in children in rural Bangladesh. *Toxicology and Applied Pharmacology*, 80-87.
- [6]. El-Mosalamy, H., Salman, T.M., Ashmawey, A.M., Osama, N. (2012). Role of E. coli infection in the process of bladder cancer - an experimental study. *Infectious Agent and Cancer*, 7(19), 1-7.
- [7]. Hooper, L.V., Littman, D.R., Macpherson, A.J. (2012). Interactions between the microbiota and the immune system. *Science*, 1268–1273.
- [8]. Hughes, R. and Rowland, I. R. (2000). Metabolic activities of the gut microflora in relation to cancer. *Microbial Ecology in Health and Disease*, 179-185.
- [9]. Isokpehi, R.D., Udensi, U.K, Simmons, S.S, Hollman, A.L., Cain, A.E., Olofinsae, S.A., Hassan, O.A., Kashim, Z.A., Enejoh, O.A., Fasesan, D.E., Nashiru, O. (2014). Evaluative Profiling of Arsenic Sensing and Regulatory Systems in the Human Microbiome Project Genomes. *Microbiology Insights*, 7, 25-34.
- [10]. Johnson, C.H., Dejea, C.M., Sears, C.L., Siuzdak, G. (2015). Metabolism Links Bacterial Biofilms and Colon Carcinogenesis. *Cell Metabolism*, 891–897.
- [11]. Oluymbo, O., Penfold, C. N., Diggle S.P. (2019). Competition in Biofilms between Cystic Fibrosis Isolates of *Pseudomonas aeruginosa* Is Shaped by R-Pyocins. *mBio*, 1-13.
- [12]. Popovic, V.B., Situm, M., Chow, C.T., Chan, L.S., Roje B., Terzic J. (2018). The urinary microbiome associated with bladder cancer. *Scientific Reports*, 1-8.
- [13]. Postler, T.S. and Ghosh, S. (2017). Understanding the Holobiont: How Microbial Metabolites Affect Human Health and Shape the Immune System. *Cell Metabolism*, 110-130.
- [14]. Salavati, S., Taylor, C. S., Harris, J. D., Paterson, G. K. (2018). A canine urinary tract infection representing the first clinical veterinary isolation of *Acinetobacter ursingii*. *New Microbe and New Infect*, 4-5.
- [15]. Shah, D.A., Wasim, S., Abdullah, F.E. (2015). Antibiotic resistance pattern of *Pseudomonas aeruginosa* isolated from urine samples of Urinary Tract Infections patients in Karachi, Pakistan. *Pak J Med Sci*, 341-345.
- [16]. Sharon, G., Garg, N., Debelius, J., Knight, R., Dorrestein, P.C., Mazmanian, S.K. (2014). Specialized metabolites from the microbiome in health and disease. *Cell Metab.*, 20(5), 719-730.
- [17]. Stolz, J.F., Basu, P., Oremland R.S. (2010). Microbial Arsenic Metabolism: New Twists on an Old Poison. *Microbe*, 5, 53-59.
- [18]. Ursell, L. K., Metcalf, J. L., Parfrey, L. W., Knight, R. (2012). Defining the human microbiome. *Nutr. Rev.*, 70 (Suppl. 1), 38–44.
- [19]. Whiteside, S.A., Razvi, H., Dave, S., Reid, G., Burton, J.P. (2015). The microbiome of the urinary tract—a role beyond infection. *Nat. Rev. Urol.*, 12, 81-90.
- [20]. Wu, P., Zhang, G., Zhao, J., Chen, J., Chen, Y., Huang, W., Zhong, J., Zeng, J. (2018). Profiling the Urinary Microbiota in Male Patients With Bladder Cancer in China. *Front Cell Infect Microbiol*, 1-10.