Adaptation of metal and antibiotic resistant traits in novel β-Proteobacterium Achromobacter xylosoxidans BHW-15

Arif Istiaq1,2, Md. Sadikur Rahman Shuvo1,2, Khondaker Md. Jaminur Rahman1, Mohammad Anwar Siddique1, M. Anwar Hossain1 and Munawar Sultana1

1 Department of Microbiology, University of Dhaka, Dhaka, Bangladesh
2 Department of Microbiology, Noakhali Science and Technology University, Noakhali, Bangladesh

ABSTRACT

Chromosomal co-existence of metal and antibiotic resistance genes in bacteria offers a new perspective to the bacterial resistance proliferation in contaminated environment. In this study, an arsenotrophic bacterium Achromobacter xylosoxidans BHW-15, isolated from Arsenic (As) contaminated tubewell water in the Bogra district of Bangladesh, was analyzed using high throughput Ion Torrent Personal Genome Machine (PGM) complete genome sequencing scheme to reveal its adaptive potentiality. The assembled draft genome of A. xylosoxidans BHW-15 was 6.3 Mbp containing 5,782 functional genes, 1,845 pseudo genes, and three incomplete phage signature regions. Comparative genome study suggested the bacterium to be a novel strain of A. xylosoxidans showing significant dissimilarity with other relevant strains in metal resistance gene islands. A total of 35 metal resistance genes along with arsenite-oxidizing aioSXBA, arsenate reducing arsRCDAB, and mercury resistance merRTPADE operonic gene cluster and 20 broad range antibiotic resistance genes including β-lactams, aminoglycosides, and multiple multidrug resistance (MDR) efflux gene complex with a tripartite system OM-IM-MFP were found co-existed within the genome. Genomic synteny analysis with reported arsenotrophic bacteria revealed the characteristic genetic organization of ars and mer operonic genes, rarely described in β-Proteobacteria. A transposon Tn21 and mobile element protein genes were also detected to the end of mer (mercury) operonic genes, possibly a carrier for the gene transposition. In vitro antibiotic susceptibility assay showed a broad range of resistance against antibiotics belonging to β-lactams, aminoglycosides, cephalosporins (1st, 2nd, and 3rd generations), monobactams and even macrolides, some of the resistome determinants were predicted during in silico analysis. KEGG functional orthology analysis revealed the potential of the bacterium to utilize multiple carbon sources including one carbon pool by folate, innate defense mechanism against multiple stress conditions, motility, a proper developed cell signaling and processing unit and secondary metabolism-combination of all exhibiting a robust feature of the cell in multiple stressed conditions. The complete genome of the strain BHW-15 stands as a genetic basis for the evolutionary adaptation of metal and the antibiotic coexistence phenomenon in an aquatic environment.
INTRODUCTION

With the passage of time, bacteria have acquired a number of mechanisms for both metal and antibiotic resistances upon evolution. The genetic plasticity of bacteria allows them to acquire such survival strategies by mutations, alteration of gene expression or genetic material acquisition which leads to the harborage of resistance determinants within (Silver & Phung, 1996; Munita & Arias, 2016).

Metals are common elements found throughout the earth’s crust naturally, and these are widely distributed in the environment (Tchounwou et al., 2012). Each metal maintains a distinct biogeochemical cycle on earth and can transfer from animal to bacteria as part of cycling processes. Some metals are essential and some are toxic to cellular system depending on metal species as well as cell type. For a bacterium, metals including nickel, iron, copper, and zinc are required as trace elements and are essential for some metabolic reactions. On the contrary, some metals such as mercury, silver and cadmium are harmful even at very low concentration and have no biological role to the organism (Hughes & Poole, 1989). Bacterial associations with metals are quite diverse and the genomic level induction for their tolerance or transformation depends on the exposure level.

On the other hand, extensive use of many antibiotics and their disposal from clinical waste and industrial origin may contaminate the water. This can act as an inducer for the dispersion of antibiotic resistance prevalence in both clinical and environmental bacteria (Salyers & Amabile-Cuevas, 1997; Walsh, 2006). Also, it can lead to a potential alteration of microbial ecosystems affecting their community composition and functions (Baquero, Martínez & Cantón, 2008).

Bacteria can acquire resistance to both metals and antibiotics simultaneously. The co-selection of these resistances can be caused by co-resistance or cross-resistance occurring through multi-resistance genetic elements such as transposon, integron, and plasmid (Baker-Austin et al., 2006). A rising concern is the development of antimicrobial resistance in metal contaminated environments (Wright et al., 2006; Matyar, Kaya & Dincer, 2008; Tuckfield & McArthur, 2008). A possible mechanism could be the selection of metal resistance by metal stress acting as a determinant for the antibiotic resistance acquisition making a bridge between non-antibiotic agents (e.g., metal) and antibiotic resistance (Summers et al., 1993). It is possible that the way of the selection of resistance variants for metal may be similar to the selection of antibiotic-resistant strains.

Bangladesh has been noted as the largest massive As poisoning occurrence country by the World Health Organization (WHO). The reason behind the exposure is for naturally occurring inorganic As accumulation in the groundwater of several colonized areas (Smith, Lingas & Rahman, 2000). Therefore bacteria colonizing such environment might have some As resistance or conversion mechanism within. Previously we found diverse arsenotrophic bacteria living in arsenic affected groundwater in different areas of...
Bangladesh (Sultana et al., 2017). Having such a metal resistant microbiome its worth studying their genomic potentiality as well as there other possible resistance mechanisms. Moreover, a study showed that metal could induce antibiotic resistance in bacteria (Chen et al., 2015). A genome-wide analysis provides an area for better understanding of possible environmental co-selection and adaptation processes in bacteria against metals and antibiotics along with unique metabolism and survival potential in stressed environment. Therefore, in this study, a draft genome of environmental strain Achromobacter xylosoxidans BHW-15 collected from As contaminated ground water is reported and analyzed thoroughly to reveal the genomic features and its innate resistance focusing on multi-metal resistance and multidrug resistance (MDR), core metabolism and adaptation potentiality. The isolate was retrieved from As contaminated tubewell water (total As content 0.01 mg/L) collected from the Bogra district of Bangladesh.

MATERIALS AND METHODS

Bacterial isolation and screening of arsenite transformation

The isolate designated as BHW-15 was retrieved from a tubewell water in Bogra District of Bangladesh on arsenite supplemented heterotrophic growth medium (Sultana et al., 2017). Arsenite transformation potential was analyzed by both KMnO₄ and AgNO₃ assay. Qualitative KMnO₄ screening method was used to determine the arsenite conversion initially (Fan et al., 2008). KMnO₄ has characteristic pink color and it is a highly oxidizing agent. A total of 500 μL culture was taken in 1.5 mL micro-centrifuge tube and 10 μL of 0.05M KMnO₄ was added and the color change was monitored. Phenotypic KMnO₄ was verified by AgNO₃ test (Salmassi et al., 2002). The isolate was streaked on heterotrophic solid medium containing two mM sodium arsenite and incubated at 30 °C. After the growth, 0.1 M of AgNO₃ solution was added to the growth plate. Formation of a brown precipitate was observed. The isolate was also analyzed for the presence of arsenite-oxidizing aioA gene by polymerase chain reaction using specific primers (Quemeneur et al., 2008) (Forward: 5′-CCACCTCTGATGCTGGGMTGYGGNTA-3′, Reverse: 5′-TGTCGGTGGCCAGATGADNCCYT-3′) followed by Sanger sequencing of the PCR product to confirm the gene sequence.

Genome sequencing and assembly

DNA from the pure culture of the isolate BHW-15 was extracted using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The quality and quantity of the extracted genomic DNA were assured by Nanodrop ND-200 (Thermo Fisher, Waltham, MA, USA) and the integrity was assured by agarose gel electrophoresis. Whole genome sequencing was performed by Ion-Torrent High Throughput Sequencing technology. Machine generated data was transferred to the Ion Torrent server where data was processed through signal processing, base calling algorithms and adapter trimming to produce mate pair reads in FASTQ format. The FASTQ reads quality was assessed by the FastQC tool (Andrews, 2010) followed by trimming of low quality reads and reads less than 200 bp using the Trimmomatic tool (Bolger, Lohse & Usadel, 2014), where quality cut off value was Phred-20. De novo assembly of the reads was
performed using SPAdes, (version 3.5.0) genome assembler (Bankevich et al., 2012). Generated assembled reads were mapped and reordered according to a reference sequence of A. xylosoxidans A8 complete genome from NCBI (accession number: NC_014640.1) by progressive Mauve algorithm in Mauve software (Darling et al., 2004).

**Identification of bacterial species**
Assembled contigs were analyzed by BLAST and the k-mer algorithm in the KmerFinder 2.0 tool to identify the bacterium at species level (Hasman et al., 2014; Larsen et al., 2014). Whole genome based phylogenetic analysis was performed using REALPHY (Bertels et al., 2014). Annotated genome comparison was performed by locally collinear block method in Mauve (Darling et al., 2004). The Plasmid Finder 1.3 tool was used for the detection of plasmid sequence contamination (Carattoli et al., 2014).

**Genome annotation, analysis and metabolic reconstruction**
The assembled draft genome of BHW-15 was annotated by multiple annotation schemes to improve accuracy. Used software includes NCBI Prokaryotic Genome Annotation Pipeline (Tatusova et al., 2016), PROKKA (e = 0.000001) (Seemann, 2014), RAST (e = 0.000001) (Aziz et al., 2008) and KAAS (Moriya et al., 2007). Annotated genes by each software were then cross checked for each tool. For the detection of tRNA and tmRNA, trNAscan (Lowe & Eddy, 1997) and Aragorn (Laslett & Canback, 2004) software were used accordingly. Secondary metabolite gene clusters were identified by anti-SMASH version 4.0.2 software (Medema et al., 2011). The SEED viewer (Aziz et al., 2012) was used for the exploration and comparative analysis of annotated genes. KEGG MGMapper tool (Kanehisa & Goto, 2000) was used for metabolic pathway reconstruction.

**Antibiotic susceptibility assay**
Antibiotic susceptibility test was conducted by Kirby–Bauer disk diffusion method (Bauer et al., 1966). A total of 14 antibiotics belonging to 10 antibiotic groups covering six different mode of action including oxacillin, ampicillin, cefalexin, cefuroxime, cefotaxime, cefepime, aztreonam, polymixin B, gentamicin, doxycycline, chloramphenicol, azithromycin, nalidixic acid, and nitrofurantoin were used for this study. Antibiotic susceptibility was interpreted referring to the CLSI guidelines and Antimicrobe Database (Roberts & Lang, 2009; Clinical and Laboratory Standards Institute, 2009).

**Accession number**
The whole genome datasets generated and analyzed during the current study are available in the NCBI GENBANK repository (accession number: PZMK0000000.1).

**RESULTS**
Whole genome sequencing of the arsenite oxidizing isolate BHW-15 identified the bacterium as A. xylosoxidans. The assembled filtered draft genome of A. xylosoxidans BHW-15 strain was 6,301,677 bp assembled into 2,049 contigs. The GC content of the genome was 65%. The genome possessed 8,159 Coding sequences (CDS) by RAST, 7,627 by NCBI, and 6,732 CDS by PROKKA. Using RASTtk (Brettin et al., 2015) annotation
scheme in the RAST software, 32% of all genes were located in the generated subsystems and the rest 50% were out of the subsystem list. The genome contained three incomplete phage site containing phage-associated phage body protein genes indicating

Figure 1 Phage gene distribution and organization within the genome. The circle (A) represents the concatenated whole reordered genome. In linear representation of regions (B–D), colored shapes indicate the relative position and type of the genes it contains. DOI: 10.7717/peerj.6537/fig-1
multiple phage confrontation (Fig. 1). The basic features of the genome are summarized in Table 1. Annotation results from subsystem and pathway reconstruction are depicted in Fig. 2. BLAST and the SEED close strain analysis found that the genome had similarity with *Bordetella* along with other *Achromobacter* strains. Kmer based genomic comparison and whole genome based phylogeny showed the *A. xylosoxidans* BHW-15 had the closest proximity to the strain “A8” (Fig. 3A). Further functional gene comparison between A8 and BHW-15 using SEED (File S1) and localized co-linear block revealed that the BHW-15 strain differs in metal resistance gene profile to A8 strain. BHW-15 possess a unique genetic island and organization of *aio* resistance island along with distinctive *ars* island that was absent in A8 strain (Fig. 3B).

### Metal resistance genes and operon clusters within the genome of isolate BHW-15

Both metal and antibiotic-resistant genes were present within the genome of BHW-15. A total of 35 metal resistance genes along with two arsenic operon gene clusters (arsenite oxidizing *aioBA* and arsenate reducing *arsRCDAB*) and a mercury resistance *merRTPCADE* operon gene cluster were present where *mer* operon is at the right end of *aio* operon gene cluster. There is a *tn21* transposon-like gene to the right end of *mer* operon gene cluster. The genome also possessed copper, zinc, and cadmium resistance-associated genes (Table 2).

*Achromobacter xylosoxidans* BHW-15 harbored two oxidase genes namely *aioA* (arsenite oxidase large subunit and *aioB* (arsenite oxidase small subunit) preceded by a phosphate transporter or inorganic arsenic binding, *aioX* (periplasmic component gene),

| Feature                                | Value                  |
|----------------------------------------|------------------------|
| Draft genome Size                      | 6,306,677              |
| GC content                             | 65%                    |
| NCBI (GeneMarkS+)                      |                        |
| Number of coding sequences (CDS)       | 7,627                  |
| Number of protein coding genes         | 5,782                  |
| Number of pseudo genes                 | 1,847                  |
| Number of RNAs                         | 125                    |
| Number of tRNAs                        | 64                     |
| Number of rRNAs                        | 7,18,32 (5S,16S,23S)   |
| Number of ncRNAs                       | 4                      |
| RAST SOFTWARE (RASTtk)                 |                        |
| Genes in subsystem                     | 2,533                  |
| Hypothetical                           | 99                     |
| Non-Hypothetical                       | 2,434                  |
| Genes not in subsystem                  | 5,626                  |
| Hypothetical gene                      | 2,102                  |
| Non-hypothetical gene                  | 3,524                  |

**Table 1** General features of the *Achromobacter xylosoxidans* BHW-15 genome.
and a sensor histidine kinase, *aioS* (a transmembrane signal transduction gene) (*Wolanin, Thomason & Stock, 2002*). Following *aioA*, there was an electron transporting Cytochrome c551/c552 gene, a Molybdenum cofactor biosynthesis gene *moaA* and an As operon repressor gene. In the island, two version of *aioA* gene were detected in two overlapping reading frames. The genome also contained a complete mercury resistance operon *merRTPADE* gene island near to the arsenite oxidizing *aio* operonic gene island. The distance between these two islands was 2,304 bp with only one gene (putative phosphatase) in-between.

The chromosome of *A. xylosoxidans* BHW-15 also possessed Arsenate reducing operon gene island *arsRCDAB* surrounded by ABC transporter and Phosphate transport
system genes. Synteny analysis with other metal converting bacteria showed that the genomic organization of these resistance island has significant distinction and content similarity with other metal resistant bacteria (Fig. 4).
Antibiotic resistance genes within the genome of isolate BHW-15

In *A. xylosoxodans* BHW-15, several antibiotic-resistant genes co-existed along with metal resistance genes. Present genes were involved in several different antibiotic resistance mechanisms by enzymatic degradation and efflux pump systems. According to RAST, the genome possessed resistance genes against β-lactams, Fluoroquinolones, and...
Using in vitro antimicrobial susceptibility tests, the bacteria showed resistance to beta-lactams from narrow spectrum to broad-spectrum penicillin, 1st generation to 3rd generation cephalosporins, aminoglycosides, monobactam, and macrolides, whereas it showed susceptibility to polymyxin B, tetracycline, nitrofuran and moderate susceptibility to 4th generation ciprofloxacin, chloramphenicol, and nalidixic acid (Table 4). These resistances suggest the possible expression of detected antibiotic resistance genes within the bacterium. Several genes for efflux systems like MDR MAR locus, multidrug-resistant tripartite system and efflux system complex OM-IM-MFP were also detected which might facilitates active efflux of antibiotic or metal. Within the genome one pili gene cluster associated with bacterial movement was also present. In addition to the metal and antibiotic resistance, these bacteria possess several genes that may be associated with its defense mechanism and bacterial pathogenesis.

### Signaling and stress response genes

**Metabolic pathway analysis**

Pathway reconstruction for the genes of A. xylosoxidans BHW-15 genome produces a number of complete and incomplete pathways (one or two blocks missing). In the genome
these detected pathways are distributed under five major KEGG pathway categories including cellular process, metabolism, environmental information processing, genetic information processing, and human diseases (Fig. 2). BHW-15 possesses several genes that are associated with different type of adaptation supporting pathways that might help the bacteria being robust in its life span. These pathways include motility, flagellar assembly, quorum sensing, biofilm formation; biosynthesis of vitamin, co-factors, folate, one carbon pool, secondary metabolites like terpinoids, polyketides etc. Moreover, several xenobiotic degradation metabolism pathways like amino benzoate degradation, cytochrome P450 were also found supported according to the genomic context.

Secondary metabolites analysis
According to AntiSMASH, the genome possesses five secondary metabolite gene clusters with 14 putative gene clusters. Detected secondary metabolites include ectoin (osmolites protective substance) (Bernard et al., 1983), resorcinol, arylopolyene (protects from reactive oxygen species) (Schöner et al., 2016), and terpene (ecological role), phosphonate (global phosphorus cycle) (Yu et al., 2013) that supports the bacteria for surviving in harsh environment (File S2).

DISCUSSION
The genome analysis helps to achieve a pertinent inference for the coexistence of metal and antibiotic resistance genes. The isolate was retrieved from an arsenic contaminated tubewell water. Therefore, it can be expected to find some sort of As resistance in the genome. In genome analysis, two As metabolizing operon like gene clusters $aioSXBA$ (oxidation) and $arsRCDAB$ (reduction) were detected. Presence of such dual system was previously reported in highly efficient arsenite oxidizing bacteria Achromobacter.

| Antibiotic group | Mode of action | Generic name | Antibiotic | Result |
|------------------|----------------|--------------|------------|--------|
| Penicillins      | B-lactamase inhibit | Oxacillin (narrow) | OX1 | R |
|                  |                | Ampicillin (broad) | AMP10 | R |
| Cephalosporin (1st G) | Cefalexin | CL 30 | R |
| Cephalosporin (2nd G) | Cefuroxime | CXM 30 | R |
| Cephalosporin (3rd G) | Cefotaxim | CTX 30 | R |
| Cephalosporin (4th G) | Cefepime | FEP 30 | MS |
| Monobactams      |                | Aztreonam | ATM 30 | R |
| Polymixin B      | Outer membrane permeability | Polymixin B | Pb300 | S |
| Aminoglycosides  | Protein synthesis (30S) | Gentamicin | Cn10 | R |
| Tetracycline     | Doxycycline | DO 30 | S |
| Chloramphenicol  | Protein synthesis (50S) | Chloramphenicol | C 30 | MS |
| Macrolides       | Azithromycin | AZM 15 | R |
| Quinolone        | DNA Topoisomerase | Nalidixic acid | NA 30 | MS |
| Nitrofurans      | DNA damage | Nitrofurantoin | F 300 | S |

Note: R, Resistant; S, Sensitive; MS, Moderately sensitive; G, Generation.
arsenitoxydans SY8 (Li et al., 2012) suggesting the A. xylosoxidans BHW-15 could also have a high efficiency in arsenite oxidation. Analysis of synteniness found that such organization of aio was quite distinctive. A possible way for such organization to function in arsenite oxidation is, AioS sense the As (III) in the environment generating proper signal to activate other genes, AioX binds and transport the arsenite into the cell, aioBA encodes the oxidase enzyme which converts arsenite to arsenate, CytC accepts the electron and transport to the cellular electron transport chain and finally the system is controlled by the arsenical resistance repressor gene in the right end of the operon. This scenario supports the aerobic respiratory oxidation of arsenite (Gihring & Banfield, 2001). In arsenate reduction by ars genes, arsC encodes the arsenate reductase and expand substrate specificity for transporting by efflux transporters ArsB, ArsR, and ArsD function as primary and secondary regulators of the ars operon accordingly, and ArsA encodes membrane-associated ATPase protein attached to ArsB energizing the efflux pump by ATP hydrolysis (Rosen, 1990; Liu & Rosen, 1997; Silver, 1998; Li et al., 2002). Such arrangement of arsenate reductase was similar to other high arsenate metabolizing bacteria. Notably, both of the aio and ars operons were similar to high arsenic transforming Herminimonas sp. While Herminimonas sp. is rare in the environment and found in highly metal contaminated zone, BHW-15 was found in groundwater but gained similar genes, indication of high arsenic resistance activity. However, how these two As oxidation-reduction systems are regulated in the bacterial cell in an aquatic environment is not clearly understood. Considering the functions and the genetic organization a possible mechanism for the regulation of these two systems can be deduced. In which, both operons can either work individually and efflux out converted arsenate (aio) and arsenite (ars) or work as a unit where environmental arsenite is converted to arsenate inside the cell by aio operonic genes and then re-converted upon expedition of deposition tolerance, to arsenite (ArsC), leading to the eviction (ArsB) from the cell by ars operon. Thus, detoxification of arsenite and arsenate is performed (Carlin et al., 1995). However, aio operonic island didn’t have any regulator of its own; therefore, these cluster might work with other functional operon to perform successfully which could be the mer operon existed just upstream to aio operon or may be with ars operon as suggested above. This mercury resistance mer operon was found juxtaposed to aio (2,304 bp gap with a putative phosphatase gene in between). At the end of mer operon, a tn21 gene was found suggesting a transposon-mediated resistance development in the bacterial genome (Cynthia, Ruth & Anne, 1999). In mer operon, merR and merD act as regulator and coregulator consecutively, merP encodes for periplasmic mercury (2+) binding protein, merA encodes for mercuric ion reductase, and merT, merC, and merE encode for mercury transporting protein (Nascimento & Chartone-Souza, 2003). Along with As and Hg, the genome also possesses resistance genes against Cu, Zn, Cd, Cr, and Co. Metals can interfere with several bacterial cellular functions like protein activity, oxidation, nutrient assimilation, membrane stability, and DNA replication (Nies, 1999; Lemire, Harrison & Turner, 2013). In response, bacteria develop metal resistance by efflux, reduction, detoxification, or biofilm formation (Harrison et al., 2008). Although, Cu and Zn are essential as trace elements others are very toxic to bacteria (Hughes & Poole, 2019).
Cu, Zn, Cd, and Cr resistance genes were found involved in similar functions for resistance mechanism such as sensing (cusR, czrR, hmhhK), efflux (ciA, clfA, czcA, cusB, chrA, corC), oxidation (mO), resistance (crB, czaC, czeD, chrB), and repressor (copG, tR, cusR, zraR).

In a similar manner, the genome harbors several antibiotic resistance genes responsible for beta-lactamase, aminoglycosides, MDR efflux pumps, and MDR tripartite system. Detected bl and blC genes encode for beta-lactamase and other penicillin binding protein protecting against beta-lactam ring inhibiting antibiotics (Neu, 1969). Also, in supportive manner, pathway analysis showed that these genes support a complete beta-lactam resistance pathway. In the antibiotic susceptibility test, the isolate showed resistance against almost all beta-lactams such as penicillin, 1st–3rd generation cephalosporin, monobactam, and moderately sensitive to the 4th generation cephalosporin. The isolate also found resistant to aminoglycosides with adaA1 and adaA2 adenyl transferase genes protecting against protein synthesis inhibition, along with moderately sensitive to chloramphenicol and quinolone. Thus, A. xylosoxidans BHW-15 has developed a multilayer resistance to membrane modification to protein-DNA synthesis inhibition by various antibiotic drugs. With some deviation these antibiotic resistance genes were also found in other A. xylosoxidans strains (Amoureux et al., 2013; Jakobsen et al., 2013).

Therefore, all these antimicrobial resistance genes might have been active within the genome that satisfy the explanation for such broad range antimicrobial resistance activity showed by the isolate. This broad metal and antibiotic resistance scenario indicates the evolutionary adaptation and resistance development of the bacteria by high selective pressure in the environment. In the genome, presence of pili genes for motility and stress tolerating genes for heat-cold shock, detoxification, osmotic pressure, carbon starvation, dormancy, etc. support the bacterial efficacy for thriving in high contamination (Stevenson, 1977; Thieringer, Jones & Inouye, 1998; Watson, Clemens & Foster, 1998; Mille, Beney & Gervais, 2005; Jones & Lennon, 2010; Maleki et al., 2016; Essa, Al Abboud & Khatib, 2018; Sun, Liu & Hancock, 2018).

In addition, three phage signatures in the chromosome also indicate bacterial adaptation and recovery against phage attack. Phage propagation in the environment can determine the host bacterial diversity and variation (Casjens, 2003; Koshella & Meaden, 2013; Parmar et al., 2017).

These three phage regions (Fig. 1) within the genome can be explained as the survival of bacteria which also leads to the bacterial robust feature (Koshella & Brockhurst, 2014). It is not known if these phage immunities have some effect with such high metal—antibiotic resistance or vice-verse but it shows a possibility that this resistance potential might somehow facilitate the bacteria to overcome phage attacks.

Hence, considering the co-existence phenomenon, some questions arise like how this bacterium could have achieved such antibiotic and metal cross resistances in the natural aquatic environment, how these two different resistance might interact with each other and is there any impact of one resistance to the regulation of the other one. A whole genome study of pathogenic strain A. xylosoxidans NH44784-1996 causing cystic fibrosis revealed almost similar metal-antibiotic resistance genes possession. But there was
a significant difference in the metal and antibiotic resistome. Such as arsenite oxidizing genes in BHW-15 were completely absent in NH44784-1996 and antibiotic resistance genes and developed resistance in NH44784-1996 was much higher than BHW-15 strain (Tables 2 and 3) (Jakobsen et al., 2013).

Therefore, a possible explanation for this co-existence might be, in the environment metal exerts a selective pressure that indirectly induces the selection of antibiotic resistance, especially in the environment contaminated with these two elements (Foster, 1983; McIntosh et al., 2008) or alternatively antibiotics might exert positive selective pressure on bacteria to acquire metal resistance, thus one resistance might have promoted the development of others leading to the coexistence phenomenon in bacteria (Baker-Austin et al., 2006).

While in the aquatic environment, antibiotics from the industrial origin and antibiotic resistance genes in pathogenic bacteria from animal origin are circulating due to excessive use of the antibiotics (Walsh, 2006). It has been reported that antibiotic disturbance in the environment affects primary microbial process such as nitrogen transformation, methanogenesis, and sulfate reduction (Ding & He, 2010). Likewise, as antibiotic interferes with the fundamental cellular process it may also impact on metal resistance gene regulation. Alternatively, the metal may also interfere with antibiotic resistance gene regulation. Therefore, the presence of antibiotic resistance genes might have impact on the metal resistance regulation or the vise verse. In the KEGG analysis, a number of cellular processes like survival and adaptation promoting pathways have been observed within the genome. It can be inferred from its genetic potential of quite developed carbon and energy metabolisms that these systems might facilitate the bacterium to utilize diverse carbon source for energy production and surviving diverse contaminated environment. Presence of the multiple stress tolerant genes and genes for defensive efflux pump, signaling, quorum sensing, flagella, anti-toxin metabolism all together gives an idea that total process in combination might be helping the bacteria to go on through resistance adaptation process. In genome comparison some arsenic related genes were not observed in the same species from different source. This indicates the existence of resistant determinant acquisition mechanism of the bacterium upon necessity. Also, genomic profile suggests a possible mobilome of metal resistance in the bacteria (As, Hg) where there was a Tn21, the flagship of transposon on the upstream of the As-Hg operons. Disease association based on the resistance genes that were found is worth to study further (Harbottle et al., 2006; Jakobsen et al., 2013; Ventola, 2015; Zaman et al., 2017). Described association also supports the bacterial pathogenicity as an opportunistic pathogen indicating it might exacerbate the disease condition of immune suppressed patient (Newman et al., 1984; Turel et al., 2013; Dupont et al., 2018).

All together the total scenario is alarming to consider the multi-potential ability of this environmental A. xylosoxidans BHW-15 and cast a new insight on metalantibiotic resistance proliferation. Further study of the other arsenic or metal metabolizing bacteria focusing on antibiotic metal cross resistance might reveal the inner mechanism and the future resistance pattern and risk to the environment.
CONCLUSIONS
Finally, our data supports the hypothesis that environmental selective pressure of antibiotic or metal from pollution can lead to the development of multi-metal and antibiotic-resistant bacteria. It also establishes a possibility for the interaction between the metal and antibiotic resistance regulation and metabolic potentiality in relation. Thus, it stands as a basis for further co-existence of resistance and metabolic potential interaction study to understand the metal-antibiotic resistance interaction in biogeochemical cycle and its impact on microbiome.

ACKNOWLEDGEMENTS
The corresponding author thanks Ms. Farzana Diba, Scientific officer in the Bangladesh Atomic Energy Commission and PhD student at the Department of Microbiology, University of Dhaka for her assistance in bacterial isolation.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding
This work was supported by the University Grants Commission (UGC) and Ministry of Science and Technology, Govt. of Bangladesh. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures
The following grant information was disclosed by the authors:
University Grants Commission (UGC).
Ministry of Science and Technology.

Competing Interests
The authors declare that they have no competing interests.

Author Contributions
• Arif Istiaq performed the experiments, analyzed the data, prepared figures and/or tables, approved the final draft.
• Md. Sadikur Rahman Shuvo performed the experiments, analyzed the data, approved the final draft.
• Khondaker Md. Jaminur Rahman performed the experiments, approved the final draft.
• Mohammad Anwar Siddique analyzed the data, approved the final draft.
• M. Anwar Hossain authored or reviewed drafts of the paper, approved the final draft, dr. Hossain provided laboratory facilities and guidance.
• Munawar Sultana conceived and designed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft, dr. Sultana managed the grant under which the research was conducted.
Data Availability
The following information was supplied regarding data availability:

Data is available at the NCBI GenBank repository, accession number PZMK00000000.1.

Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.6537#supplemental-information.

REFERENCES
Amoureux L, Bador J, Fardeheb S, Mabille C, Couchot C, Massip C, Salignon A-L, Berlie G, Varin V, Neuwirth C. 2013. Detection of *Achromobacter xylosoxidans* in hospital, domestic, and outdoor environmental samples and comparison with human clinical isolates. *Applied and Environmental Microbiology* 79(23):7142–7149 DOI 10.1128/AEM.02293-13.

Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. Available at https://www.bioinformatics.babraham.ac.uk/projects/fastqc/ (accessed 2 September 2017).

Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsmas K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil JK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* 9(1):75 DOI 10.1186/1471-2164-9-75.

Aziz RK, Devoid S, Disz T, Edwards RA, Henry CS, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Stevens RL, Vonstein V, Xia F. 2012. SEED servers: high-performance access to the SEED genomes, annotations, and metabolic models. *PLOS ONE* 7(10):e48053 DOI 10.1371/journal.pone.0048053.

Baker-Austin C, Wright MS, Stepanauskas R, McArthur JV. 2006. Co-selection of antibiotic and metal resistance. *Trends in Microbiology* 14(4):176–182 DOI 10.1016/j.tim.2006.02.006.

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology* 19(5):455–477 DOI 10.1089/cmb.2012.0021.

Baquero F, Martinez J-L, Cantón R. 2008. Antibiotics and antibiotic resistance in water environments. *Current Opinion in Biotechnology* 19(3):260–265 DOI 10.1016/j.copbio.2008.05.006.

Bauer AW, Kirby WM, Sherris JC, Turck M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology* 45(4_ts):493–496 DOI 10.1093/ajcp/45.4_ts.493.

Bernard T, Jebbar M, Rassouli Y, Himdi-Kabbab S, Hamelin J, Blanco C. 1983. Ectoine accumulation and osmotic regulation in *Brevibacterium linens*. *Journal of General Microbiology* 139(1):129–136 DOI 10.1099/00221287-139-1-129.

Bertels F, Silander OK, Pachkov M, Rainey PB, Van Nimwegen E. 2014. Automated reconstruction of whole-genome phylogenies from short-sequence reads. *Molecular Biology and Evolution* 31(5):1077–1088 DOI 10.1093/molbev/msu088.

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimer for Illumina sequence data. *Bioinformatics* 30(15):2114–2120 DOI 10.1093/bioinformatics/btu170.

Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M. 2015. RASTtk: a modular and extensible implementation of the RAST
algorithm for building custom annotation pipelines and annotating batches of genomes. *Scientific Reports* 5:8365 DOI 10.1038/srep08365.

Carattoli A, Zankari E, Garcia-Fernandez A, Voldby Larsen M, Lund O, Villa L, Moller Aarestrup F, Hasman H. 2014. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrobial Agents and Chemotherapy* 58(7):3895–3903 DOI 10.1128/aac.02412-14.

Carlin A, Shi W, Dey S, Rosen BP. 1995. The ars operon of *Escherichia coli* confers arsenical and antimonial resistance. *Journal of Bacteriology* 177(4):981–986 DOI 10.1128/jb.177.4.981-986.1995.

Casjens S. 2003. Prophages and bacterial genomics: what have we learned so far? *Molecular microbiology* 49(2):277–300 DOI 10.1046/j.1365-2958.2003.03580.x.

Chen S, Li X, Sun G, Zhang Y, Su J, Ye J. 2015. Heavy metal induced antibiotic resistance in bacterium LSJC7. *International journal of molecular sciences* 16(10):23390–23404 DOI 10.3390/ijms161023390.

Clinical and Laboratory Standards Institute. 2009. Performance Standards for Antimicrobial Susceptibility Testing: Nineteenth Informational Supplement. CLSI document M100–S19. Wayne: Clinical and Laboratory Standards Institute.

Cynthia AL, Ruth MH, Anne OS. 1999. Transposon Tn21, flagship of the floating genome. *Microbiology and Molecular Biology Reviews* 63(3):507–522.

Darling AC, Mau B, Blattner FR, Perna NT. 2004. Mauve: multiple alignment of conserved genomic sequence with rearrangements. *Genome Research* 14(7):1394–1403 DOI 10.1101/gr.2289704.

Ding C, He J. 2010. Effect of antibiotics in the environment on microbial populations. *Applied Microbiology and Biotechnology* 87(3):925–941 DOI 10.1007/s00253-010-2649-5.

Dupont C, Jumas-Bilak E, Doisy C, Aujoulat F, Chiron R, Marchandin H. 2018. Chronic airway colonization by *Achromobacter xylosoxidans* in cystic fibrosis patients is not sustained by their domestic environment. *Applied and Environmental Microbiology* 84(23):e01739-18 DOI 10.1128/AEM.01739-18.

Essa AM, Al Abboud MA, Khatib SI. 2018. Metal transformation as a strategy for bacterial detoxification of heavy metals. *Journal of Basic Microbiology* 58(1):17–29 DOI 10.1002/jobm.201700143.

Fan H, Su C, Wang Y, Yao J, Zhao K, Wang Y, Wang G. 2008. Sedimentary arsenite-oxidizing and arsenate-reducing bacteria associated with high arsenic groundwater from Shanyin, Northwestern China. *Journal of Applied Microbiology* 105(2):529–539 DOI 10.1111/j.1365-2672.2008.03790.x.

Foster TJ. 1983. Plasmid-determined resistance to antimicrobial drugs and toxic metal ions in bacteria. *Microbiological Reviews* 47(3):361–409.

Gihring TM, Banfield JF. 2001. Arsenite oxidation and arsenate respiration by a new *Thermus* isolate. *FEMS Microbiology Letters* 204(2):335–340 DOI 10.1111/j.1574-6968.2001.tb10907.x.

Harbottle H, Thakur S, Zhao S, White DG. 2006. Genetics of antimicrobial resistance. *Animal Biotechnology* 17(2):111–124 DOI 10.1080/10495390600957092.

Harrison JJ, Turner RJ, Joo DA, Stan MA, Chan CS, Allan ND, Vrionis HA, Olson ME, Ceri H. 2008. Copper and quaternary ammonium cations exert synergistic bactericidal and antibiofilm activity against *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy* 52(8):2870–2881 DOI 10.1128/AAC.00203-08.

Hasman H, Saputra D, Sicheritz-Ponten T, Lund O, Svendsen CA, Frimodt-Moller N, Aarestrup FM. 2014. Rapid whole-genome sequencing for detection and characterization of
microorganisms directly from clinical samples. *Journal of Clinical Microbiology* 52(1):139–146 DOI 10.1128/jcm.02452-13.

**Hughes MN, Poole RK. 1989.** The functions of metals in micro-organisms. In: Hughes MNP, Poole RK, eds. *Metals and Microorganisms*. London: Chapman and Hall, 1–38.

**Jakobsen TH, Hansen MA, Hansen PO, Hansen L, Riber L, Cockburn A, Kolpen M, Hansen CR, Ridderberg W, Eickhardt S. 2013.** Complete genome sequence of the cystic fibrosis pathogen *Achromobacter xylosoxidans* NH4784-1996 complies with important pathogenic phenotypes. *PLOS ONE* 8(7):e68484 DOI 10.1371/journal.pone.0068484.

**Jones SE, Lennon JT. 2010.** Dormancy contributes to the maintenance of microbial diversity. *Proceedings of the National Academy of Sciences of the United States of America* 107(13):5881–5886 DOI 10.1073/pnas.0912765107.

**Kanehisa M, Goto S. 2000.** KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Research* 28(1):27–30 DOI 10.1093/nar/28.1.27.

**Koskella B, Brockhurst MA. 2014.** Bacteria–phage coevolution as a driver of ecological and evolutionary processes in microbial communities. *FEMS Microbiology Reviews* 38(5):916–931 DOI 10.1111/1574-6976.12072.

**Koskella B, Meaden S. 2013.** Understanding bacteriophage specificity in natural microbial communities. *Viruses* 5(3):806–823 DOI 10.3390/v5030806.

**Larsen MV, Cosentino S, Lukjancenko O, Saputra D, Rasmussen S, Hasman H, Sicheritz-Ponten T, Aarestrup FM, USSery DW, Lund O. 2014.** Benchmarking of methods for genomic taxonomy. *Journal of Clinical Microbiology* 52(5):1529–1539 DOI 10.1128/jcm.02981-13.

**Laslett D, Canback B. 2004.** ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. *Nucleic Acids Research* 32(1):11–16 DOI 10.1093/nar/gkh152.

**Lemire JA, Harrison JJ, Turner RJ. 2013.** Antimicrobial activity of metals: mechanisms, molecular targets and applications. *Nature Reviews Microbiology* 11(6):371–384 DOI 10.1038/nrmicro3028.

**Li X, Hu Y, Gong J, Lin Y, Johnstone L, Rensing C, Wang G. 2012.** Genome sequence of the highly efficient arsenite-oxidizing bacterium *Achromobacter arsenitoxydans* SY8. *Journal of Bacteriology* 194(5):1243–1244 DOI 10.1128/JB.06667-11.

**Li S, Rosen BP, Borges-Walmsley MI, Walmsley AR. 2002.** Evidence for cooperativity between the four binding sites of dimeric ArsD, an As(III)-responsive transcriptional regulator. *Journal of Biological Chemistry* 277(29):25992–26002 DOI 10.1074/jbc.M201619200.

**Liu J, Rosen BP. 1997.** Ligand interactions of the ArsC arsenate reductase. *Journal of Biological Chemistry* 272(34):21084–21089 DOI 10.1074/jbc.272.34.21084.

**Lowe TM, Eddy SR. 1997.** tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Research* 25(5):955–964 DOI 10.1093/nar/25.5.955.

**Maleki F, Afra Khosravi AN, Taghinejad H, Azizian M. 2016.** Bacterial heat shock protein activity. *Journal of Clinical and Diagnostic Research* 10(3):BE01 DOI 10.7860/JCDR/2016/14568.7444.

**Matyar F, Kaya A, Dincer S. 2008.** Antibacterial agents and heavy metal resistance in Gram-negative bacteria isolated from seawater, shrimp and sediment in Iskenderun Bay, Turkey. *Science of The Total Environment* 407(1):279–285 DOI 10.1016/j.scitotenv.2008.08.014.

**McIntosh D, Cunningham M, Ji B, Fekete FA, Parry EM, Clark SE, Zalinger ZB, Gilg IC, Danner GR, Johnson KA, Beattie M, Ritchie R. 2008.** Transferable, multiple antibiotic and mercury resistance in Atlantic Canadian isolates of *Aeromonas salmonicida* subsp. *salmonicida* is associated with carriage of an IncA/C plasmid similar to the *Salmonella enterica* plasmid pSN254. *Journal of Antimicrobial Chemotherapy* 61(6):1221–1228 DOI 10.1093/jac/dkn123.
Medema MH, Blin K, Cimermancic P, De Jager V, Zakrzewski P, Fischbach MA, Weber T, Takano E, Breitling R. 2011. antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. *Nucleic Acids Research* 39(suppl_2):W339–W346 DOI 10.1093/nar/gkr466.

Mille Y, Beney L, Gervais P. 2005. Compared tolerance to osmotic stress in various microorganisms: towards a survival prediction test. *Biotechnology and Bioengineering* 92(4):479–484 DOI 10.1002/bit.20631.

Moriya Y, Itoh M, Okuda S, Yoshizawa AC, Kanehisa M. 2007. KAAS: an automatic genome annotation and pathway reconstruction server. *Nucleic Acids Research* 35(Web Server):W182–W185 DOI 10.1093/nar/gkm321.

Munita JM, Arias CA. 2016. Mechanisms of antibiotic resistance. *Microbiology Spectrum* 4(2):481–511.

Nascimento AM, Chartone-Souza E. 2003. Operon mer: bacterial resistance to mercury and potential for bioremediation of contaminated environments. *Genetics and Molecular Research* 2(1):92–101.

Neu HC. 1969. Effect of beta-lactamase location in *Escherichia coli* on penicillin synergy. *Applied Microbiology* 17(6):783–786.

Nies DH. 1999. Microbial heavy-metal resistance. *Applied Microbiology and Biotechnology* 51(6):730–750 DOI 10.1007/s002530051457.

Parmar KM, Gaikwad SL, Dhakephalkar PK, Kothari R, Singh RP. 2017. Intriguing interaction of bacteriophage-host association: an understanding in the era of omics. *Frontiers in Microbiology* 8:559 DOI 10.3389/fmicb.2017.00559.

Quemeneur M, Heinrich-Salmeron A, Muller D, Lievremont D, Jauzein M, Bertin PN, Garrido F, Joulain C. 2008. Diversity surveys and evolutionary relationships of *aob* genes in aerobic arsenite-oxidizing bacteria. *Applied and Environmental Microbiology* 74(14):4567–4573 DOI 10.1128/AEM.02851-07.

Roberts SA, Lang SDR. 2009. *Achromobacter (Alcaligenes) Species.* Available at http://www.antimicrobe.org/b18rev.asp.

Rosen BP. 1990. The plasmid-encoded arsenical resistance pump: an anion-translocating ATPase. *Research in Microbiology* 141(3):336–341 DOI 10.1016/0923-2508(90)90008-E.

Salmassi TM, Venkateswara K, Satomi M, Newman DK, Hering JG. 2002. Oxidation of Arsenite by *Agrobacterium albertimagni*, AOL15, sp. nov., isolated from hot creek, California. *Geomicrobiology Journal* 19(1):53–66 DOI 10.1080/014904502317246165.

Salyers AA, Amabile-Cuevas CF. 1997. Why are antibiotic resistance genes so resistant to elimination? *Antimicrobial Agents and Chemotherapy* 41(11):2321–2325 DOI 10.1128/aac.41.11.2321.

Schöner TA, Gassel S, Osawa A, Tobias NJ, Okuno Y, Sakakibara Y, Shindo K, Sandmann G, Bode HB. 2016. Aryl Polyenes, a highly abundant class of bacterial natural products, are functionally related to antioxidative carotenoids. *ChemBioChem* 17(3):247–253 DOI 10.1002/cbic.201500474.

Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30(14):2068–2069 DOI 10.1093/bioinformatics/btu153.

Silver S. 1998. Genes for all metals—a bacterial view of the periodic table. The 1996 Thom Award Lecture. *Journal of Industrial Microbiology and Biotechnology* 20(1):1–12 DOI 10.1038/sj.jim.2900483.
Silver S, Phung LT. 1996. Bacterial heavy metal resistance: new surprises. *Annual Review of Microbiology* 50(1):753–789 DOI 10.1146/annurev.micro.50.1.753.

Smith AH, Lingas EO, Rahman M. 2000. Contamination of drinking-water by arsenic in Bangladesh: a public health emergency. *Bulletin of the World Health Organization* 78(9):1093–1103.

Stevenson LH. 1977. A case for bacterial dormancy in aquatic systems. *Microbial Ecology* 4(2):127–133 DOI 10.1007/bf02014283.

Sultana M, Mou TJ, Sanyal SK, Diba F, Mahmud ZH, Parvez AK, Hossain MA. 2017. Investigation of Arsenotrophic microbiome in arsenic-affected Bangladesh groundwater. *Groundwater* 55(5):736–746 DOI 10.1111/gwat.12520.

Summers AO, Wireman J, Vimy MJ, Lorscheider FL, Marshall B, Levy SB, Bennett S, Billard L. 1993. Mercury released from dental “silver” fillings provokes an increase in mercury- and antibiotic-resistant bacteria in oral and intestinal floras of primates. *Antimicrobial Agents and Chemotherapy* 37(4):825–834 DOI 10.1128/AAC.37.4.825.

Sun E, Liu S, Hancock RE. 2018. Surfing motility: a conserved yet diverse adaptation among motile bacteria. *Journal of Bacteriology* 200(23):e00394-18 DOI 10.1128/JB.00394-18.

Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Research* 44(14):6614–6624 DOI 10.1093/nar/gkw569.

Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. 2012. Heavy metal toxicity and the environment. *Experientia Supplementum* 101:133–164 DOI 10.1007/978-3-7643-8340-4_6.

Thieringer HA, Jones PG, Inouye M. 1998. Cold shock and adaptation. *BioEssays* 20(1):49–57 DOI 10.1002/(SICI)1521-1878(199801)20:1<49::AID-BIES8>3.0.CO;2-N.

Tuckfield RC, McArthur JV. 2008. Spatial analysis of antibiotic resistance along metal contaminated streams. *Microbial Ecology* 55(4):595–607 DOI 10.1007/s00248-007-9303-5.

Turel O, Kavuncuoglu S, Hosaf E, Ozbek S, Aldemir E, Uygur T, Hatipoglu N, Siraneci R. 2013. Bacteremia due to *Achromobacter xylosoxidans* in neonates: clinical features and outcome. *Brazilian Journal of Infectious Diseases* 17(4):450–454 DOI 10.1016/j.bjid.2013.01.008.

Ventola CL. 2015. The antibiotic resistance crisis: part 1: causes and threats. *Pharmacy and Therapeutics* 40(4):277–283.

Walsh TR. 2006. Combinatorial genetic evolution of multiresistance. *Current Opinion in Microbiology* 9(5):476–482 DOI 10.1016/j.mib.2006.08.009.

Watson SP, Clements MO, Foster SJ. 1998. Characterization of the starvation-survival response of *Staphylococcus aureus*. *Journal of Bacteriology* 180(7):1750–1758.

Wolanin PM, Thomason PA, Stock JB. 2002. Histidine protein kinases: key signal transducers outside the animal kingdom. *Genome Biology* 3(10):reviews3013.1 DOI 10.1186/gb-2002-3-10-reviews3013.

Wright MS, Peltier GL, Stepanauskas R, McArthur JV. 2006. Bacterial tolerances to metals and antibiotics in metal-contaminated and reference streams. *FEMS Microbiology Ecology* 58(2):293–302 DOI 10.1111/j.1574-6941.2006.00154.x.

Yu X, Doroghazi JR, Janga SC, Zhang JK, Circello B, Griffin BM, Labeda DP, Metcalf WW. 2013. Diversity and abundance of phosphonate biosynthetic genes in nature. *Proceedings of the National Academy of Sciences of the United States of America* 110(51):20759–20764 DOI 10.1073/pnas.1315107110.

Zaman SB, Hussain MA, Nye R, Mehta V, Mamun KT, Hossain N. 2017. A review on antibiotic resistance: alarm bells are ringing. *Cureus* 9:e1403 DOI 10.7759/cureus.1403.