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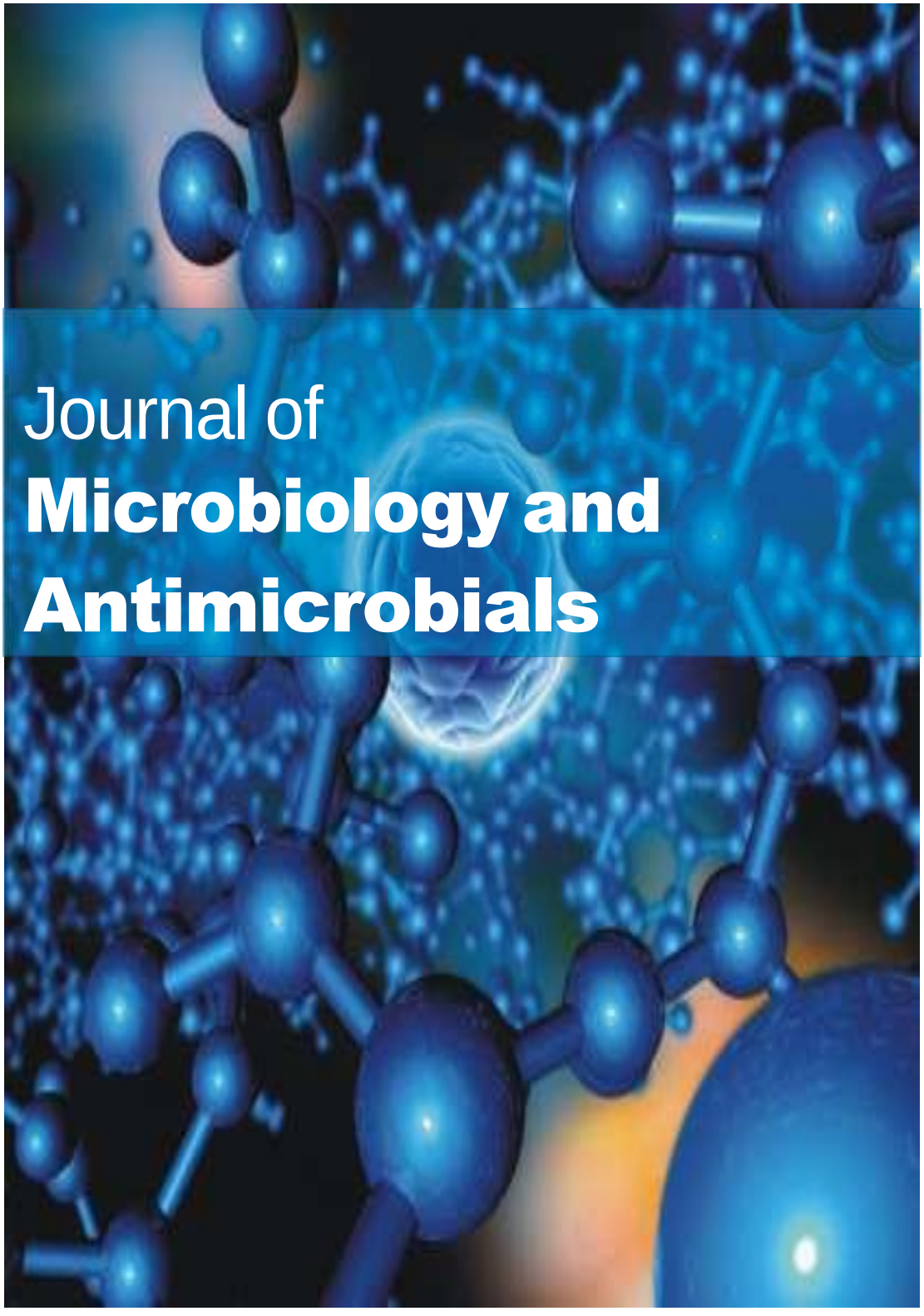

Journal of Microbiology and Antimicrobials

July-December 2023
ISSN 2141-2308
DOI: 10.5897/JMA

ACADEMIC JOURNALS

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Vol. 15(2), pp. 12-22, July-December 2023
DOI: 10.5897/JMA2023.0463
Article Number: 1C5331671367
ISSN: 2141-2308

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Journal of Microbiology and Antimicrobials

Review

Universal stress protein (*usp*) gene role: A conceptual hotspot for general resistance and microbial survival

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Received 25 July, 2023; Accepted 20 September, 2023

The increasing trend of antimicrobial resistance is quite alarming and requires urgent response to circumvent the menace. Microorganisms are known to be resistant to adverse reactions using different survival strategies. Indiscriminate usage of antibiotics has driven the rapid spread of antibiotic resistance within pathogenic and opportunistic microorganisms. The majority of microbes have established defence mechanisms against antibiotics, including the efflux of antibiotics from cells via efflux pumps, enzymatic destruction of the antibiotic molecules, and chemical changes that protect the cellular targets of antibiotics. But in the course of this review highlight, we found microbial-resistance approach to antibiotics by stress-mediated process. The concepts of resilience and resistance are complementary and both represent different aspects of the stability of ecosystems. Recently, most stress conditions have been advocated to be molecular-switches to *usp* gene expression, which support microbial survival. It has been established that microorganisms resist macrophage-phagocytosis and such resistance was attributed to *usp* gene expression. This showed that *usp* acts as an essential linkage to resistance of various antimicrobial agent. The ubiquitous nature of *usp* in various organisms was found to help organisms survive under stress conditions. Furthermore, this review will help explore the extent to which *usp* gene expression provides resilience and resistance to microorganisms.

Key words: Universal stress protein, resilience, antimicrobial resistance.

INTRODUCTION

Most microorganisms survive stressful conditions through various known and unknown mechanisms. More often, microorganisms can adapt to stress conditions through various mRNA regulation methods and protein control translation with other general stress response procedures (Fang et al., 2017). Microbial stress response contributes to the high rate of microbial resistance with potential targets on infectious diseases, which are traceable to universal stress proteins (*usp*) gene expression. According to O'Connor and McClean (2017), *usp*s are conserved categories of stress proteins within the range of 140 to 160 amino acids that are found in living things

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including prokaryotes and eukaryotes (O'Connor et al., 2020).

The decreasing effect of antibiotics against bacterial infections globally is alarming and has been attributed to misuse and abuse of antibiotics over the last 50 years. Some other school of thought attributed resistance to prevalence and stability nature of resistance genes among bacterial pathogens which resulted to emergence of multidrug resistant pathogens (Akgul et al., 2018). The discovery of resistance genes among bacterial pathogen, enabled scientists to understand some microbial resistance mechanisms, yet the full procedure is still not fully understood. Hence, this calls for more novel antibiotics and chemotherapeutic approach, to help circumvent the menace attributed to microbial resilience and resistance. Moreover, some recent research work revealed that universal stress protein affect microbial latency, resilient and microbial resistance to stress (Jia et al., 2016; Akram et al., 2021).

Universal stress proteins comprise a group of gene that are induced by different stress conditions (Hassan et al., 2021) which sometimes relapses after stressors were withdrawn (Nachin et al., 2005). The *usp* constitutes a family of stress-induced gene that encompassed a conserved gene group that can be found in bacteria, eukaryotes and other higher animals (Liu et al., 2007; Gomes et al., 2011). This genetic sequence that translates into cytoplasmic proteins, identified to be *usp*, whose expression cushion a wide range variety of internal and external stresses. The level of the *usp* gene expressed on most isolates has been attributed to be as a result of variety of stress conditions, which include starvation, temperature, oxidative stress, high salt concentration, pH, ethanol, and antibiotics (Gustavsson et al., 2002). As microbes actively grow within and outside of their hosts as well as when they are in exponential and stationary phase, microorganisms have a broad variety of coping strategies to deal with the constant onslaught of stress situations. According to their genomic sequences, these isolates have a homologue that is a member of the family of universal stress proteins (Kvint et al., 2003; Liu et al., 2007; Hassan et al., 2021).

Microorganism *usp* is made of a natural biological defence mechanism, which helps confer resistance to external factors under stress (Hassan et al., 2018). The organism's ability to survive in such extreme environments were obtained by the expression of *usp* through mechanism not fully understood. The *usp* gene has been demonstrated to facilitate the spread of pathogens to their hosts as shown by Rayan and Ray (2004) and Hensel (2009). Study conducted by Hingley-Wilson et al. (2010) stated that the expression of *usp* genes in *Mycobacterium tuberculosis* enables the pathogenic isolate survive during treatment. Study conducted on *usp M. tuberculosis* showed that *usp* gene when expressed to stress helps the isolate enter the latency phase during tuberculosis infection. But *usp* gene expressed by many microorganisms such as *Listeria, Acinetobacter* and *Salmonella* species were found to enable survival even against phagocytes. Moreover, *usp* gene expression in *Pseudomonas aeruginosa* and *Porphyromonas gingivalis* is the reason for its resistance and microbial resilience through biofilm formation (Drumm et al., 2009).

Microbial protein adhesion found in *Burkholderia cepacia* and *Staphylococcus aureus* was found to play this specific role recently as *usp* gene is expressed. There are findings indicating that *usp* neutralizes some antibiotic effects causing resistance and its mechanism of action may be of help to enable design better functional antimicrobial (O'Connor and McClean, 2017). Similar work done by Liu et al. (2007) showed that *usp* when expressed played a formidable role in the presence of some microorganisms such as *Salmonella* and *Staphylococcus* species growth and virulence factor production.

These *usp* genes were characterized with the presence of a gene motif conserved within a domain which was present in organisms and was highly induced under certain stress as first observed in *Escherichia coli usp*A (Nachin et al., 2005; Siegele, 2005; Jia et al., 2016). It was observed in *E. coli* that *usp*A deletion arrests growth at the stationary phase and are likely to initiate early death phase. Nowadays, numerous *usp* in a given microbial species, possess paralogous *usp* genes. Work done by Nachin et al. (2005) showed that elimination of *usp* genes exert different physiological approaches, showing that they have different role responses to microorganisms (Diez et al., 2000).

Based on *usp* similarity of the *usp* domain, one tandem-type gene, namely *usp*E, the *usp* gene family in *E. coli* was separated into two sub-families *usp*E1 and *usp*E2, based on its functionality (Hafeez et al., 2021). *Nitrosomonas europea* and *Archaeoglobus fulgidus* were found to have six and eight known copies of genes encoding for different *usp* genes respectively, while *Arabidopsis thaliana* had four distinct copies of the *usp* genes (Tkaczuk et al., 2013). According to Nachin et al. (2005), the *usp* genes in *E. coli* produce proteins that are involved in oxidative stress, adhesion, and motility, among other things.

Expression of *usp* genes was suggested to be regulated by sigma (σ) factors within RNA polymerases, and polyphosphates or guanosine 5'-diphosphate 3'-diphosphate (ppGpp) were another important regulator of *usp* (Kvint et al., 2003). Under stressed conditions, *usp* genes were found to be expressed which aid microbial survival under harsh conditions (Tkaczuk et al., 2013), even though the full mechanisms were not fully understood.

Universal stress proteins were found to be ubiquitously expressed in microorganisms with the general property as adaptation of bacteria to oxidative stress, temperature, hypoxia variation and some other stress. There are

Figure 1. *Salmonella typhimurium's usp* phylogenetic tree. Clustal Omega and T-REX web servers were used to build the tree. A similar tree was found when the E. coli USPs were analysed (Gustavsson et al., 2002), with the exception of uspD's presence in the branch that included UspA and UspC (Bangera et al., 2015).

evidences which show that *usp* gene facilitates microbial colonisation and pathogenicity to the human host environment, which enhances human host mortality rate (O'Connor and McClean, 2017).

Universal stress protein classification and some functions

At the Sanger Centre, universal stress proteins have been categorized into a fast-expanding orthologous grouping called Pfam (Gustavsson et al., 2002; Mistry et al., 2021). The *usp* domain can be found in more than 1,000 different proteins that function either by overlapping or a single (Bateman et al., 2004; Isokpehi et al., 2021).

All spheres of life contain members of the *usp* protein family. Furthermore, bacteria with the *usp* gene typically have many copies of it. Additionally, the *usp* comes in a variety of forms. One is a collection of tiny *usp* proteins with one *usp* domain that is between 14 and 15 kDa, and the other is a bigger version that has two *usp* domains in tandem and weighs around 30 kDa (Gustavsson et al., 2002).

Based on comparable sequences in the *usp* domain-1, *usp*F and *usp*G belong to a distinct subfamily than *usp*A, *usp*C, and *usp*D which are members of the same related subfamily as shown in Figure 1. According to Gustavsson et al., 2002) and Bangera et al. (2015), the tandem-type *usp*E protein's *usp* domain-2 is more closely connected to the *usp*F and *usp*G subgroups.

Usp gene class I comprise *usp*A*, usp*C, and *usp*D genes that can exist in single or even in overlapping nature. Gustavsson et al. (2002) in their research finding towards effect of oxidative stress, found that if *E. coli* is challenged with hydrogen peroxide, *usp*ACD gene expression are found to increase at the exponential growth phase (Hafeez et al., 2021).

Nachin et al. (2005) also challenged *E. coli* with superoxide-generating agent like premium motor spirit and it was found to confer resistance against oxidative stress. The *usp*A and *usp*D appearance involved in altering cell capacity to resist oxidative agents but *usp*D alone helps regulate intracellular iron availability (Isokpehi et al., 2021). The *usp*F and *usp*G, which were determined to be mostly connected to adhesion and motility, are part of the Class II *usp* gene. They appeared to play a small function in the resistance to oxidative stress.

Class III/IV *usp* genes function mainly by overlapping the role of all the other classes where *usp*E involve in regulating the cell capacity to withstand oxidative stress defence which is obtainable in class-I and also regulates cells aggregation that is obtainable in class-II (Nachin et al., 2005). *Usp*E is a tandem *usp* that probably developed as a result of a gene-splicing event. In the past, *usp*E domain-1 and *usp*E domain-2 were names for the two different usp domains that it contained. When *usp*E proteins are divided into smaller pieces and treated individually, the *usp*E2 domain is more closely connected to *usp*FG. This is demonstrated by both the clustering analysis displayed in Figure 2, where *usp*E1 groups are more closely associated to class-I-*usp*.

An intended class V protein that was projected to be *usp*B integral membrane was inserted as an out group to make it easier to separate the non-membrane *usp* families because it is thought to be member of protein and not a true universal stress protein (Lougheed et al., 2022). These *usp* genes, during expression induce microbial resilient or resistant to the organism against the induced stress.

Resilience and resistance response to microbial system

For the sake of human health, it is crucial to comprehend

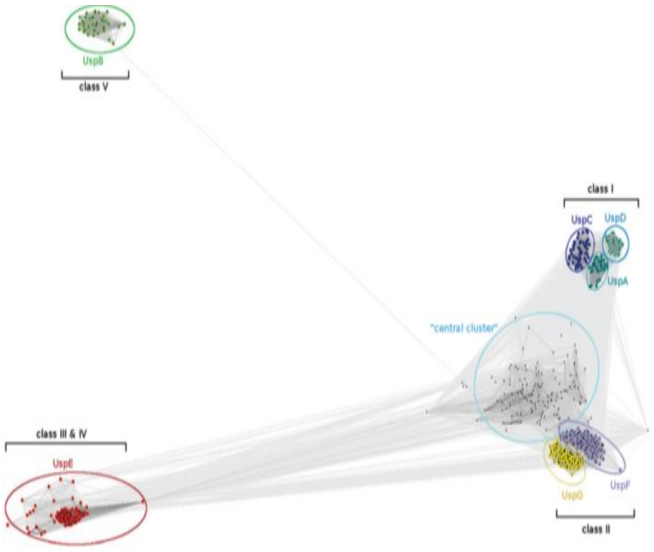

Figure 2. Clustering analysis results. Results of the clustering analysis with complete length, displaying the results of the clustering with each Usp separated into distinct domains and handled independently. Each usp family is shown in a different color and is labeled (Tkaczuk et al., 2013).

the mechanisms governing microbiome stability in the face of ongoing disruption. According to Sommer et al. (2017), perturbation or stress is an event with variable magnitude, rhythmicity, and context that can have an impact on the immediate environment directly or indirectly by eliciting a subsequent reaction.

Perturbations are classified depending on duration (Walker et al., 2004). In general, stress responses are classified as either short-term or long-term or even continuous event (Yang et al., 2019). Some organism gave rise to resilience response whereas others cause resistance in nature when exposed to stress. In order to understand the effect of stress in microbial system, it is important to survey microbial resilience and resistance. The system ability remains stable in the presence of a disturbance or stress is referred to as resistance (Oliver et al., 2015), whereas resilience refers to the amount of stress that a system can withstand before shifting towards a new equilibrium with a potential function or service (Gunderson, 2000; Lozupone et al., 2012).

According to Gunderson (2000) and Shade et al. (2012), microbial resilience is a complicated characteristic of a system made up of multiple essential parts. First of all, resistance is a crucial component of resilience since it represents the likelihood that an organism or plant may depart from its constant state (Yang et al., 2019).

Second, the latitude of change may be described as the greatest degree to which a microorganism can be altered by a stress before it loses the ability to return to the initially stable form (Gustavsson et al., 2002). In facing a long-termed or continuous stress pattern, like non-persisted nutrient availability, ethanol, oxidative stress, high salt concentration and antibiotics exposure (David et al., 2014), new approach has been understood, whose mechanism of action is traceable to *usp-gene* mechanism (Luo et al., 2023) (Figure 3).

Antibiotic stress: A condition for microbial resilience and resistance

Now that more bacterial strains are becoming resistant to antibiotics, their use was continually put to the test. Nanduri et al. (2008) and Woodford and Ellington (2007) discovered that universal stress protein gene expression, horizontal gene transfer, and mutations related to bacterial DNA replication have all contributed to the development of the resistance. In analysing different gene expression as it concerned sub-MIC antibiotics under *Pasteurella multocida* by Nanduri et al. (2008), its Pm70 protein was found to be expressed as its compensatory response to antibiotic. Tkaczuk et al. (2013) discovered likely protein from different microorganisms under different stressors as universal stress protein.

Even though the use of antibiotics for treatment cannot be ignored, yet the use of antibiotics has a significant impact on the human microbiota. Despite some research suggesting that these effects are only temporary (Subramanian et al., 2014), other research indicates that these effects are permanent and disrupt homeostasis pathways that regulate immune responses (Dethlefsen and Relman, 2011). Whether an antibiotic-

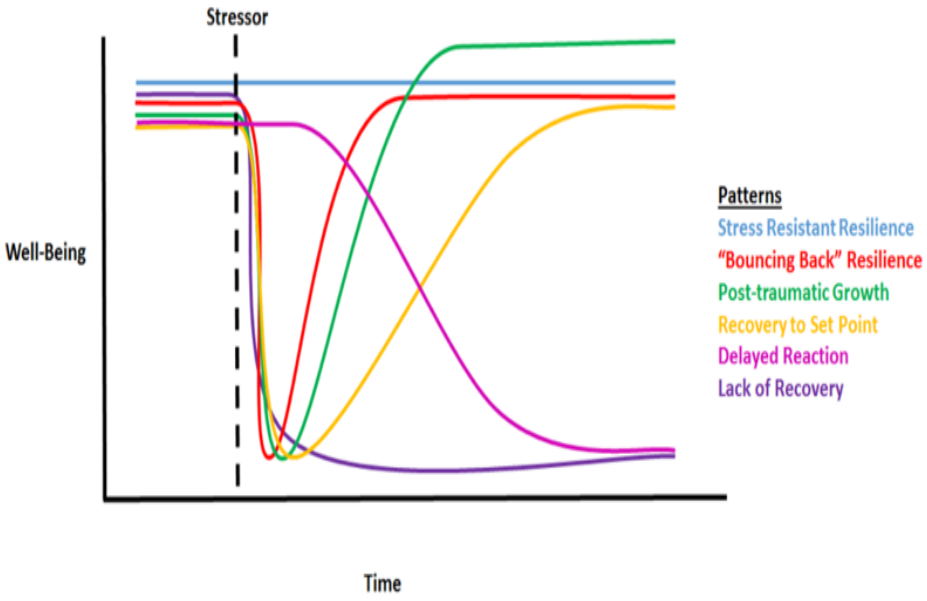

Figure 3. Patterns of stress responses. In considering microbial well-being or responses to different stressors, it has been discovered that stress can cause a change in behaviour of microorganisms. Whereas others may recover with time after stress has been removed, some may not recover from stress with time even when the stress has been withdrawn (Shade et al., 2012). This behavioural attitude can be attributed to usp-gene expression.

mediated stress response in a microorganism became resistant or resilient during or after stress, depends on some gene expression make-up. It is still unknown if all of the microbiota impacts that have been noticed were due to antibiotics' direct action or whether some of them are as a result of secondary effects physiochemical parameters or immunological responses (Sommer et al., 2017).

The resiliency of the microbiota may also influence how well a patient responds to antibiotic therapy. Resilience phenomena following antibiotic perturbation in humans have been studied in several observational studies. Dethlefsen and Relman (2011) showed that in-take of ciprofloxacin for five days with human volunteers displayed abrupt changes in the distal gut of microbiome's makeup. Studies conducted by Jernberg et al. (2007) revealed that *Phocaeiola* species resisted clindamycin treatment within microbiome niche that was not recovered after antibiotic treatment withdrawal. In contrast, *Phocaeiola dorei* were recovered and was attributed *usp* gene expression within *P. dorei* (Dethlefsen and Relman, 2011).

The number of *Lactobacillus* species within the small intestines and the expression of antimicrobial peptides gene were initially reduced by the regularly prescribed antibiotic amoxicillin (Schumann et al., 2005). Incomplete resilience was suggested to be caused by short-term use of antibiotic, which altered not only the mucosal antibody repertoire but also the composition of the microbiota in almost all participants within the case-study healthy volunteers. This work suggested that a key mechanism for direct antibody-dependent systemic immunological activities interaction between intestinal mucosal immune cells and the disturbed non-resilient microbiota (Jernberg et al., 2007) needed to be discovered.

Antibiotics have been discovered to be a stressor, which can cause permanent-resilience, also known as resistance or partial-resilience, also known as resistance to the antibiotic therapy. *Clostridium difficile* infection is an example of clinical microbial resilience and it exclusively expresses its stress gene on administration of broad-spectrum antibiotic (Chang et al., 2008). It was established that little stress from commensal microorganisms gave *C. difficile* infection an advantage which allows pathogenic isolates to develop microbial resistance through stress-gene expression.

Intriguingly, a recent study showed that antibiotic causes reduction of the microbiota particularly causes a decrease in the generation of secondary metabolites, which facilitates its colonization (Theriot et al., 2016). Even though varieties of mechanisms to resist stress conditions during active growth in the host have not been fully understood but *usp*-gene expression is another 'window of opportunity' to explore and cushion the effect of antimicrobial resistance. A homologous to the family of *usp* gene was observed in the genome sequence of some isolates (Liu et al., 2007) (Figure 4).

Antimicrobial resistance: Its relationship to *usp*-gene expression

Antibiotic resistance to bacterial infectious disease is an increasing trend that threatens public health

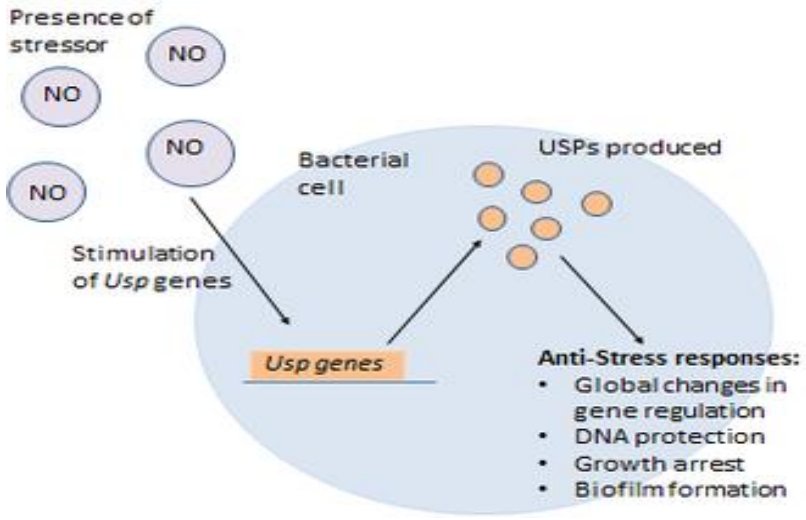

Figure 4. *Usp* gene expression response in bacterial cell. The presence of nitric oxide (NO), stimulates the expression of usp gene within the cell thereby translating into proteins that enable the bacteria cell to withstand the effect of the stress.

(Bandyopadhyay and Mukherjee, 2020). Obolski and Hadany (2012) research found evidence that certain bacteria are more likely to undergo horizontal gene transfer and mutation under stressful settings, leading to an earlier onset of antibiotic resistance. But recent findings have shown that bacterial exposure to stressful condition acquires resistance mainly by *usp*-gene expression technique (Handel et al., 2016).

It was hypothesized that the gene should correspond to UDP-(3-O-(R-3-hydroxymyristoyl))-N-acetyl glucosamine after looking at the *Proteus mirabilis* universal stress protein (Pm-*usp*) structure. The *E. coli* proteins that contain the ligand - *lpx*A as was cited by Williams and Raetz (2007) and *lpx*C as was cited by Clayton et al. (2013) and its deacetylated by-products, function as catalysts for the initial step in the synthesis of lipid-A. The lipopolysaccharide membrane-anchoring moiety that makes up Gram-negative bacteria's outer membrane is known as lipid-A. The fact that this membrane protects the bacterium from harmful antibacterial agents makes it essential for bacterial survival (Arabia et al., 2021) Therefore, the initiation and expression of Pm-*usp* by the antibiotic-stressor, helps the isolate to resist the antimicrobial compound (Nanduri et al., 2008). If the presence of antimicrobial compound can mediate antimicrobial resistance through *usp* gene expression, it will be needful to understand the mechanism of action so as to help combat resistance menace of useful antibiotics.

Usp appears to be a biological defence mechanism, which help microorganism pose resistance to external factors (Masamba and Kappo, 2021). Under stress conditions, *usp* can be overexpressed at persistent stress exposure and through varieties of mechanisms which aid microorganisms survive such harsh conditions (Lee et al., 2022). It has been demonstrated that *usp* aid pathogens in establishing a foothold within host organisms (Rayan and Ray, 2004; Hensel, 2009) and that *usp* expression enhances microbial defences, opening the door to pathogenic infection and difficult treatment due to resistance (Liu et al., 2007). *M. tuberculosis* may depend on *usp* genes for intracellular survival, according to research by Hingley-Wilson et al. (2010). An increasing group of tiny cytoplasmic proteins that have been influenced by a wide range of stimuli that makes up the universal stress protein family (Kvint et al., 2003; Gustavsson et al., 2002). *E. coli* K-12 *usp*A was discovered as a stress-mediated gene in 1992 after demonstrating that its synthesis was stimulated in response to many stress shocks (Nystrom and Neidhardt, 1992, 1993).

Subsequent work on *usp* gene regulation indicated that *usp* in stress responses showed that cold-shock stress does not induce synthesis of *usp* (Nachin et al., 2005), but was later disproved. It was shown that the reason why the cold-shock stresses were unable to induce *usp* gene expression in that experimental isolate was based on *usp* gene knockout.

E. coli usp gene was found to be first discovered as universal stress protein and characterized by the existence of a conserved gene motif that is present in species and is significantly elevated under specific stress (Siegele, 2005; Jia et al., 2016). It was observed that *usp* deletion in *E. coli* causes growth retardation at the stationary phase. In a given species of *E. coli,* there are typically numerous universal stress proteins paralogous. Individual *usp* gene deletion has demonstrated that these genes play distinct roles in cellular stress response (Diez

et al., 2000; Nachin et al., 2005).

Majority of species have several copies of the *usp* paralogous, varying in number according to the organism. Six genes from the *usp* family, two of which are classified as subfamilies based on how closely their *usp* domain sequences match and one tandem-type gene, *usp*E, are present in *E. coli* (Hafeez et al., 2021). In comparison to the four copies of *usp* genes found in *A. thaliana*, *N. europea* and *A. fulgidus* have six and eight copies of the *usp* genes, respectively (Tkaczuk et al., 2013).

Recent research has revealed that *usp* paralogous act to protect DNA from UV damage in response to high amounts of cytoplasmic polyphosphates or guanosine 5'-diphosphate 3'-diphosphate (ppGpp) (Gustavsson et al., 2002; Ye et al., 2020). The production of the protein occurs in response to growth inhibition brought on by a deficiency in carbon, nitrogen, sulphate, or phosphate, osmotic shock, a high pH, temperature variation, or the presence of heavy metals, or antibiotics (Yohannes et al., 2004). UspA undergoes serine and threonine phosphorylation after entering the stationary phase (Liu et al., 2007). While *usp*A overproduction results in a continuous growth-arrest state, mutants without *usp*A experience premature death during stasis. The ppGpp activates *usp*A transcriptionally while *fad*R suppresses it, knowing that *usp*A is a member of a protein superfamily that is highly conserved to be expressed via stress and can be supressed with a feedback inhibition of anti-stressor (Ye et al., 2020).

Mechanism and synthesis of *usp* gene family

Universal stress proteins are synthesize in response to growth inhibition caused by various stressors such as starvation of essential nutrient, amino acid, exposure to heat, oxidants, metals, ethanol and antibiotics (Gomes et al., 2011; Masamba and Kappo, 2021). Upon receiving environmental stress signal the *usp* gene is phosphorylated from stressor signal at both serine and threonine phosphorylation sites and sometime upon microbial entry into the stationary phase (Freestone et al., 1997; Lee et al., 2022). Mutant devoid of *usp* gene mostly do not survive stress on receiving stress signal and such stressor always cause premature death during stasis whereas overexpression of *usp* gene promotes survival in the presence of stress (Diez et al., 2000; Diao et al., 2023).

The *usp*A genes were also hypothesized to be a component of the *rec*A-dependent DNA protection (Diez et al., 2000) and repair mechanism since its absence increases vulnerability to UV radiation exposure (Liu et al., 2007). RecA and ppGpp were found to promote the transcription of several stress-related genes, while *fad*R and *fts*K were shown to suppress it (Farewell et al., 1996; Diez et al., 2000; Diao et al., 2023). However, *fad*R and *Fts*K are not involved in the induction of *usp*A during physiological stress, according to Phadtare and Inouye (2001). The *csp*C and *csp*E were found to increase the stability of *usp*A gene and make the gene expression more steadfast. An open reading frame upstream of *uspA* in *E. coli* encodes a 14 kDa protein that can withstand the majority of stressors, whereas *usp*B was discovered to withstand only ethanol stress. *Usp*B expresses via sigma S-dependent rather than sigma 70-dependent, as is the case with *uspA* (Liu et al., 2007).

The over-expression of the *rel*A gene, which linked the activation of *usp* genes caused an ectopic rise in ppGpp levels development within the cell. Mutation in the *fts*K gene can cause gene expression increase, and this super-induction could be stopped by deactivating *rec*A. This suggests that *usp* paralogues were found as control of microbial stress conditions (Gustavsson et al., 2002; Dutta et al., 2021).

Guanosine 5'-diphosphate 3'-diphosphate (ppGpp) is linked to antimicrobial drug resistance. Trimethoprim, gentamicin, and polymyxin B are more effective against ppGpp-deficient *E. coli* mutants (Greenway and England, 1999). In accordance to Pomares et al. (2008), greater ppGpp accumulation in mutant *E. coli* is associated with both increased resistance to fluoroquinolones and increased survivability in the presence of the peptide antibiotic microcin J25. Moreover, *Streptomyces coelicolor* finding suggested that the synthesis of ppGpp has recently been related to resistance to vancomycin and bacitracin (Poole, 2012).

Roles of *usp* gene in microbial stress tolerance

It has been described that *usp* gene might be linked with a wide number of act, like oxidative stress resistance, invasion, adhesion, antibiotic resistance and motility as were observed in *E. coli, M. tuberculosis, Klebsiella pneumonia,* and *P. aeruginosa* (Nachin et al., 2005; Boes et al. 2006; Tkaczuk et al., 2013; Havis et al., 2019). Most cells express many proteins necessary for their survival when under environmental stress. These proteins are typically referred to as *usp*s and the majority of them are cytoplasmic proteins, while some of them may also have enzyme-like properties (Gustavsson et al., 2002). Even so, it is still unclear exactly which biochemical processes these stress proteins fully participate in and how their cellular defense works. However, the essence of these proteins are eminent in nature and drastically found to reduce stress tolerance of microorganism when present (Bangera et al., 2015).

Methanococcus jannaschii and *Haemophilus influenza* proteins with *usp*-like domains have very similar folding configurations (Zarembinski et al., 1998), as demonstrated by the structures of these proteins (Sousa and McKay, 2001). Furthermore, structural and biochemical studies have revealed that *usp* gene domains can be of two groups namely; the ATP binding

ups and the non-ATP binding *usp* gene (Sousa and McKay, 2001).

The *M. jannaschii* (MJ0577) ATP binding protein has a triphosphate binding motif made up of three glycine residues sandwiched between two to nine amino acid residues, with a serine or threonine or asparagine coming after the last glycine. Other ATP-binding *usp* also contain the phosphate binding loop (Bangera et al., 2015). These proteins' ability to modify the conformation of the phosphate binding loop may be crucial for carrying out a variety of cellular tasks, including the regulation of ATP hydrolysis.

The *usp*F gene structure suggests that this tight ATP binding moiety conservation is not necessary but because of the stiffness of the phosphate binding loop brought on by chloride ion binding (Ye et al., 2020). This *usp*F active ATPase after chloride ion binding residues were altered thereby increasing the loop's flexibility and enabling it to adopt the cationic active conformation (Bangera et al., 2015). A pathogenic bacterium must have experienced high environmental pressures at different times during its life cycle in order for it to infect a host and cause disease. For instance, *S.* Typhimurium, the agent responsible for salmonellosis causes diarrhoea when exposed to a hostile environment. Upon entering the host cell, overcome the immune response of the host, which makes the pathogen withstand extreme stress and establish infection (Bangera et al., 2015; Dutta et al., 2021).

Microbial tolerance to stress was found to be initiated by *usp-gene* expression. The procedure was observed in studies demonstrated using wild-type and mutated *E. coli usp*A gene, which tolerate the growth of *E. coli* of the wild-type upon long term exposure to external stresses (Bandyopadhyay and Mukherjee, 2022) against mutated-type. It showed that *usp gene* expression helps in microbial tolerance to stress responses. Additionally, *usp* plays a crucial role in the virulence of *M. tuberculosis* and *Salmonella* Typhimurium C5 (Drumm et al., 2009; Liu et al., 2007), and numerous genes encoding *usp* gene similar to USPs were predicted to exist by studying the *Salmonella* spp. genome. Now in order to understand the detailed mechanism of *usp*, more research work must be done within this research area to enable researchers have an in-depth knowledge of *usp* genes analysis.

Oxidative stress: A condition for microbial resilience and resistance

According to Rahman et al. (2012), oxidative stress is a condition where the amount of oxidation (reactive oxygen species) in the body outweighs the antioxidant systems within the body system. This results in a loss of equilibrium alongside the free radicals production and its intermediates system activity, which may outweigh the system's capacity to neutralize and eliminate free radicals. In addition to dangerous occurrences like lipid peroxidation and oxidative stress also affect physiologic adaption processes and controls signal transduction (Lougheed et al., 2022). It is generally established that oxidative stress contributes in the aetiology of serious illnesses such atherosclerosis, hypertension, diabetes mellitus, ischemic disorders, and cancer, where oxygen free radicals attack biological molecules thereby causing those diseases (Yoshikawa and Naito, 2002).

Free radicals are atoms with unpaired electrons, which is typically unstable and highly reactive because unpaired electrons have a tendency to pair with other electrons. When digested *in vivo,* an oxygen molecule (O^2) goes through a four-electron reduction. By excitation of electrons that interact with transition elements during this process, reactive oxygen metabolites are produced. The reactive oxygen metabolites created in this way are known as active oxygen species and are more reactive than the original oxygen molecule.

Free radicals are the only active oxygen species with an unpaired electron. Mechanism to eliminate these extremely reactive active oxygen species is necessary for aerobic organisms to maintain existence. It has been observed that oxidative stress may stimulate the expression of some *usp* gene, which promotes microbial resistance to stress (Havis et al., 2019). This implies that *usp* expressed genes are liable to most stress resistant. Therefore, *usp*-modulator and novel antioxidant should be develop to combat the menace of incessant microbial-resistant (Rahman et al., 2012; Havis et al., 2019).

Oxidative stress: A biological modulator and a signalling molecule for *usp* gene expression

Oxidative stress not only causes cytotoxicity, but it also has a significant impact on messengers that control survival-critical processes of cell membranes (Liu et al. 2017). The changed redox status within the cell causes a number of protein kinases to be activated, which has a variety of physiological impacts (Yang et al., 2019). These protein kinases include a number of receptor and non-receptor tyrosine kinases, protein kinase C, and the MAP kinase cascade (Lougheed et al., 2022). These protein kinases are crucial for a variety of cellular processes, like activation, proliferation, and differentiation. As illustrated in Figure 5, it has been determined that *usp*A and *usp*D expression and presence confer resistance to oxidative stress on developing *E. coli* cells when they are exposed to hydrogen peroxide (Nachin et al., 2005).

CONCLUSION

The increasing knowledge of *usp* gene expression among microorganisms have programmed the cells

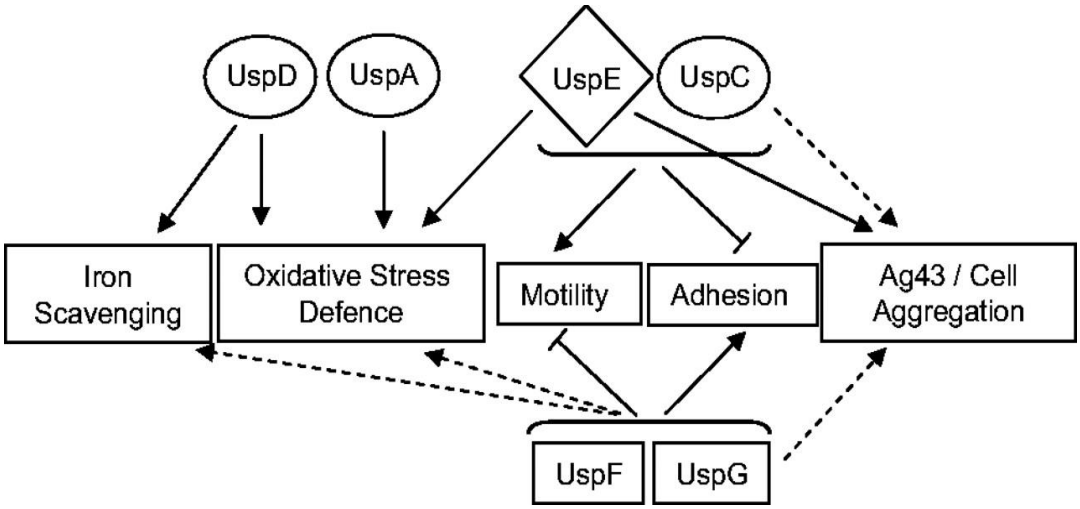

Figure 5. *E. coli usp* gene role in oxidative stress defence. Every usp has a shape around its name that denotes the class to which it belongs where circle stands for class I, a square for class II, and a diamond for classes III and IV. Arrows indicate how the usp protein affects a specific function, whereas 'T' shapes indicate how the usp protein affects other activities. The significant and minor effects of the usp in the various functions are depicted by solid and dotted lines, respectively. The inclusion of both of the aforementioned proteins is indicated as a component of the stated process by the brackets. For instance, adhesion and motility are both negatively affected by uspC and uspE, respectively (Nachin et al., 2005).

toward resisting and escaping intimidating stressors, even though the functions of *usp* genes were not fully understood (Nachin et al., 2005). It is needful now to understand the in-depth details of *usp* mechanism which will enable researcher in influencing *usp* gene expression (Poole, 2012; Dhanyalakshmi and Nataraja, 2021).

An in-depth significance of *usp* gene expression connection mediated by stress was found to cause resistance to external environmental factors (Bandyopadhyay and Mukherjee, 2020; Cui et al., 2021; Bhuria et al., 2022; Diao et al., 2023). The identification of the stress-induced effectors that promote resistance will help determine genes involvement and will also require to fully comprehend the significance and mechanism of stress responses. Further research on *usp*-modulation as therapeutic approach is required to circumvent the antimicrobial resistance architecture.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank Professor Obi Njoku, Director, South-East Zonal Biotechnology Centre, University of Nigeria, Nsukka for providing all the internet and ICT facilities needed for the research and the UNN, Department of Microbiology Post Graduate (PG) Coordinator, Professor V. N. Chigor and all the PG Staff for their administrative and technical input in this research.

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Vol. 15(2), pp. 23-28, July-December 2023
DOI: 10.5897/JMA2023.0462
Article Number: 70C806971488
ISSN: 2141-2308

http://www.academicjournals.org/JMA

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Journal of Microbiology and Antimicrobials

Full Length Research Paper

Characterization of class 1, 2 and 3 integrons in multidrug-resistant *Escherichia coli* isolated from clinical samples from Niamey, Niger

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Received 21 July, 2023; Accepted 17 October, 2023

Antibiotic resistance is a major public health problem worldwide. *Escherichia coli* is one of the bacteria most frequently isolated in hospital infections and became more resistance to common antibiotics used. This resistance to antibiotics could be attributed to a modification of the genetic supports or the acquisition of mobile genetic elements. A total of 195 multi-drug resistant *E. coli* isolated from clinical samples, were analyzed. Of these multi-drug resistant *E. coli*, 54 isolates were producing extended-spectrum beta-lactamase. The presence of class 1, 2, and 3 integrons was performed using simple PCR. To highlight the different classes of integrons, genomic DNA was extracted with the QIAmp, DNA mini, and Qiagen kit. The result of the 195 isolates DNA amplification showed that 60.5% isolates were positive for the class 1 integron, while class 2 integron was found in 6 isolates (3.1%) and class 3 integron was found in 24 isolates (12.3%). Among multi-drug resistant *E. coli* producing extended spectrum beta-lactamase, 68.5% carried the class 1 integron, 3.7% for the class 2 integron, and 13% for the class 3 integron. The results of this study showed the presence of three classes of integrons in several clinical isolates of multi-drug resistant *E. coli*. The simultaneous presence of resistance genes and integron classes in several extended-spectrum beta-lactamase-producing isolates demonstrates the need for increased monitoring of antibiotic use.

Key words: Integron, multi-drug resistant, *Escherichia coli*, extended-spectrum beta-lactamase.

INTRODUCTION

The increasing of resistance to commonly applied antimicrobial agents is being reflected by growing multiple drug resistance in bacteria and is becoming a growing threat to public health. The use of antimicrobial agents in animal husbandry has been linked to the development and spread of resistant bacteria (Agyare et

al., 2018).

Escherichia coli, a conditional pathogen, is one of the most common and important pathogens in medical care settings. It is the most prominent cause of diarrhea, urinary tract infections, septicemia, and various other clinical infections, including neonatal meningitis (Wu et al., 2021). The problem of bacterial antibiotic resistance is one of the World Health Organization's highest priorities when it comes to threats to human health (Nasif et al., 2022). Beta-lactamase mediated resistance in *E. coli* is a significant problem that requires immediate attention (Tewari et al., 2022).

Acquiring mobile elements, including plasmids, transposons, and integrons among Gram-negative bacteria, plays an important role in the development of antibiotic resistance (Sütterlin et al., 2020). Various classes of integrons possessing a wide variety of gene cassettes are distributed in bacteria throughout the world. The role of integrons as mobile genetic elements playing a central role in antibiotic resistance has been well studied and documented. Integrons are the ancient structures that mediate the evolution of bacteria by acquiring, storing, disposing, and resorting to the reading frameworks in gene cassettes (Sabbagh et al., 2021).

Several classes of integron have been described, including classes 1 and 2 of the most common integrons of multi-drug resistant. Gram-negative bacteria are associated with antibiotic treatment failure (Kaushik et al., 2018).

The presence of integrons in the clinical *E. coli* isolates is also highly related to antibiotic resistance, class 1-integron was highly prevalent in these pathogenic isolates (Nasif et al., 2022). Class I integrons of *E. coli* strains were present in all sources, while the prevalence of intI2 was lower but remarkable in food isolates (Etayo et al., 2018).

The percentage of clinical multi-drug resistant *E. coli* isolates was higher among those positive for integron II gene followed by integron III gene (Taha et al., 2018).

The gene *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{OXA}$, and *bla*$_{CTX-M}$ as well as integrons (*Int*1, *Int2*, and *Int3*) are involved in the antibiotic resistance of diarrheagenic *E. coli* (Dembélé et al., 2022).

This study aims to determine the prevalence of class 1, 2, and 3 integrons in multidrug-resistant *E. coli* isolated from the clinical specimen in two hospitals in Niamey, Niger.

MATERIALS AND METHODS

Study design and samples

It is a cross-sectional study conducted in two hospitals of Niamey, Niger (National and AMIROU BOUBACAR DIALLO hospitals). The study investigated 195 isolates of multi-drug resistant *E. coli* obtained from various clinical specimens collected from March 2014 to June 2016. The clinical specimens included: urine, stool, blood, vaginal swab, and pus.

Isolation, identification, antimicrobial susceptibility testing of isolates, and phenotypic characterization of extended-spectrum beta-lactamases (ESBL) were described in our previous study (Alio et al., 2017).

Genomic DNA extraction

Genomic DNA extraction was performed with the QIAmp, DNA mini kit (Qiagen Germany). Two colonies of *E. coli* isolates were suspended in 180 µl ATL buffer for the first digestion. The mixture was homogenized, then 20 µl of proteinase K was added, vortexed, and incubated at 56°C. After 1 h of incubation, the tube was centrifuged for 1 min at 8,000 rpm. After, 200 µl of AL buffer was added. The mixture was homogenized and incubated at 70°C for 10 min. Then 200 µl of 100% ethanol was added. The mixture was centrifuged at 8,000 rpm for 3 min. The tube containing 600 µl of the total mixture was placed in the Qiagen column and centrifuged at 8000 rpm. After 3 min, 500 µl of AW1 buffer was added to the column and centrifuged at 8000 rpm for 3 min. Once this step was complete, 500 µl of buffer AW2 was added to the column and centrifuged at 14,000 rpm for 3 min. The column was then placed in an Eppendorf tube and 200 µl of buffer AE was added. The Eppendorf tube was incubated at room temperature for 1 min and then centrifuged at 8000 rpm for 3 min. The column was then discarded, and the Eppendorf tube DNA was stored at -20°C for integron analysis.

Characterization of integrons

The presence of class 1, 2, and 3 integrons was tested using simple PCR according to Ploy et al. (2000). Primers sequences and amplicons of the different classes of integrons are listed in Table 1.

Single PCRs were performed with a final reaction volume of 25 µl. The PCR mix contained 2.5 µl of 10 X GC buffer, 0.5 µl of dNTPs (10 mM), 2 µl of $MgCl_2$ (25 mM), 0.25 µl of Taq Polymerase (5 U/l), 14.25 µl of H_2O, 1.5 µl of Forward primers, 1.5 µl of Reverse primers and 2.5 µl of DNA lysate. The PCR conditions were 94°C for 5 min, followed by 35 cycles of 94°C for 30 s for denaturation, annealing at 60°C (IntI1) and 62°C (IntI2 and IntI3) for 1 min, and then extension at 72°C for 1 min followed by a final extension of 72°C for 7 min. Amplicons were stored at 4°C for electrophoretic separation. After PCR, 10 µl of each amplicon was mixed with a drop of blue loading buffer and then separated by electrophoresis on agarose gel (1%) with tris borate EDTA buffer (1X) at 130 V and 300 mA during 1 h.

Ladder of 100 and 200 bp (HyperLadder I, Bioline) were used. Once migrated, ethidium bromide gels were visualized under UV light. The molecular weight of the amplified fragment was checked against the expected fragment using several ladders. For the positive control, DNAs from the reference strains R3 and R7 were used for class 1 and 2 integrons, respectively.

Data analysis

Data were processed and analyzed using Microsoft Excel 2013 and

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Table 1. Primers used for the detection of integrons.

Integrons	Primer sequence (5'-3')	Amplicon size (PB)	Annealing temp. (°C)	References
IntI1	F: ATTTCTGTCCTGGCTGGCGA R: ACATGTGATGGCGACGCACGA	600	60	Ploy et al. (2000)
IntI2	F: CACGGATATGCGACAAAAAGGT R: GTAGCAAACGACTGACGAAATG	806	62	Ploy et al. (2000)
IntI3	F: GCCCCGGCAGCGACTTTCAG R: ACGGCTCTGCCAAACCTGACT	600	62	Ploy et al. (2000)

Table 2. Prevalence of class 1, 2 and 3 integrons among MDR *E. coli*.

Integrons class n (%)	Isolates origin				
	Stool N=49	Urine N=134	Pus N=7	Blood N=4	Vaginal swabs N=1
IntI1	44 (89.8)	68 (50.7)	4 (57)	2 (50)	0 (0)
IntI2	2 (4.1)	3 (2.2)	1 (14.3)	0 (0)	0 (0)
IntI3	24 (49)	0 (0)	0 (0)	0 (0)	0 (0)

Med Cal version 11.0.1.0. $p < 0.05$ was considered to be statistically significant.

RESULTS

Bacterial isolates and antimicrobial susceptibility testing

A total of 195 multi-drug resistance (MDR) *E. coli* were collected and analysed during the study period. Among these isolates, 54 (27.7%) were extended-spectrum beta-lactamases producers. Therefore, 49 (25.1%) strains of multi-resistant *E. coli* were isolated from stool samples, 134 (68.7%) strains from urine samples, 7 (3.6%) from pus samples, 4 (2.1%) from blood samples, and one strain from vaginal swabs.

As shown in our previous study, high resistance to beta-lactams was observed, mainly with ampicillin (100%), amoxicillin + clavulanic acid (93.1%), cephalothin (98.2%), cefotaxime (92.6%), ceftazidime (97.2%), and ceftriaxone (83.9%) as compared to quinolone with ofloxacin (77.4%), ciprofloxacin (84.9%), and nalidixic acid (91.2%). Resistance to the monobactams was 77.4% to aztreonam, and the sulphonamides were 95.4% to trimethoprim-sulfamethoxazole (Alio et al., 2017).

Prevalence of class 1, 2 and 3 integrons in multidrug-resistant *E. coli* isolates

The PCR amplification results showed that, of the 195 isolates, 118 were positive for the class 1 integron (*IntI1*) which represented 60.5% of all tested strains while class 2 Integron (*IntI2*) was found in 6 isolates (3.1%) and the class 3 integron (*IntI3*) was found in 24 isolates (12.3%) (Table 2).

The results in Table 2 indicated a higher prevalence of *IntI1* in stool isolates (89.8%) than in other isolates from urine (50.7%), pus (57%), and blood (50%) ($p = 0.0006$).

In contrast, the prevalence of *IntI2* observed in pus isolates (14.3%) was higher than that observed in stool isolates (4.1%) and urine isolates (2.2%) ($p = 0.0020$).

On the other hand, results of this study reported the presence of *IntI3* only in stool isolates with a prevalence of 49%. Figure 1 shows amplicons sizes of the different classes of integrons.

Prevalence of class 1, 2, and 3 integrons in ESBL-producing *E. coli* isolates

Among the multidrug resistant *E. coli* isolates, 54 of them were producing extended spectrum beta-lactamases.

From stools samples, the results indicate that there was no significant difference ($p = 0.7637$) between the prevalence of *IntI1* in ESBL-producing *E. coli* (85.7%) and that observed in multidrug-resistant *E. coli* strains that did not express ESBL (91.4%). No ESBL-producing *E. coli* contained *IntI2* gene was observed. However, a prevalence of 5.7% of these integrons was observed in *E. coli* which does not express ESBL. Moreover, for *IntI3*, a prevalence of 50 and 48.6% was observed in ESBL-producing and non-ESBL-producing *E. coli* isolates, respectively ($p = 1.00$).

In urine samples, the prevalence of *IntI1* was 59.5% in ESBL-producing *E. coli* and 47.4% in multidrug-resistant *E. coli* which do not express ESBL ($p = 0.2460$). The

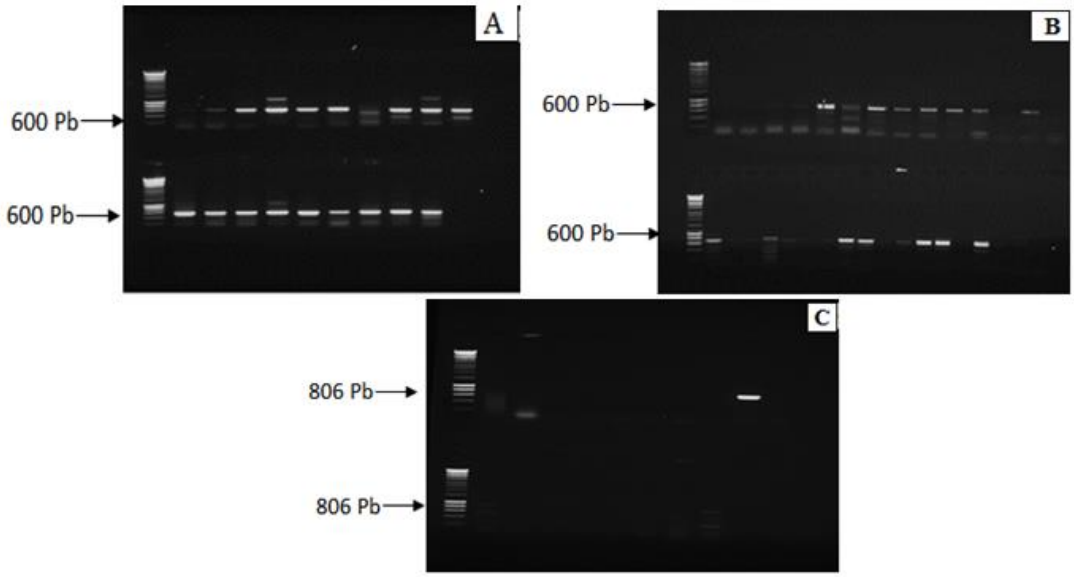

Figure 1. Integrons class *IntI1* (A), *IntI2* (C) and *IntI3* (B) of stool samples gel on agarose.

Table 3. Prevalence of class 1, 2, and 3 integrons in ESBL-producing and non-producing *E. coli* isolates.

Integrons class	Isolates origin									
	Stools		Urine		Pus		Blood		Vaginal swabs	
	ESBL + N=14	ESBL - N=35	ESBL + N=37	ESBL - N=97	ESBL + N=2	ESBL - N=5	ESBL + N=1	ESBL - N=3	ESBL + N=0	ESBL - N=1
IntI1 n (%)	12 (85.7)	32 (91.4)	22 (59.5)	46 (47.4)	2 (100)	2 (40)	1 (100)	1 (33.3)	0 (0)	0 (0)
IntI2 n (%)	0 (0)	2 (5.7)	2 (5.4)	1 (1.0)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)
IntI3 n (%)	7 (50)	17 (48.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table 4. Combination prevalence of different resistance integron classes.

Integrons class	Isolates origin				
	Stools N=49	Urine N=134	Pus N=7	Blood N=4	Vaginal swabs N=1
IntI1 + *IntI2*	2 (4.1)	2 (1.2)	0 (0)	0 (0)	0 (0)
IntI1 + *IntI3*	24 (49)	0 (0)	0 (0)	0 (0)	0 (0)
IntI2 + *IntI3*	2 (4.1)	0 (0)	0 (0)	0 (0)	0 (0)
IntI1 + *IntI2* + *IntI3*	1 (2.0)	0 (0)	0 (0)	0 (0)	0 (0)

prevalence of *IntI2* was 5.4 and 1% in ESBL-producing *E. coli* and non-ESBL-producing *E. coli*, respectively (p = 0.2207). No *IntI3* was detected in urine isolates. Only *IntI1* in ESBL-producing isolates from pus and blood was detected with a prevalence of 100% (Table 3).

Combination of different resistance integron classes

Results in Table 4 indicated that only isolates from stool and urine carry two or three classes of integrons simultaneously. In stool isolates, the prevalence of *IntI1* + *IntI3* (49%) was significantly higher (p < 0.0001) than the other types of combinations *IntI1* + *IntI2* (4.1%) and *IntI2* + *IntI3* (4.1%). However, the combination of all three integron classes (*IntI1* + *IntI2* + *IntI3*) was only observed in stool isolates with a prevalence of 2%. For urine isolates, only a prevalence of 1.2% of *IntI1* + *IntI2* was observed.

Table 5. Prevalence of isolates harbouring integron classes and resistance genes.

Integrons class	Isolates origin												
	Stools				Urine			Pus			Blood		
	bla TEM	*bla* CTX-M	*bla* OXA-1	*bla* SHV	*bla* TEM	*bla* CTX-M	*bla* OXA-1	*bla* TEM	*bla* CTX-M	*bla* OXA-1	*bla* TEM	*bla* CTX-M	*bla* OXA-1
IntI1 n (%)	42 (95.5)	31 (70.5)	33 (75)	8 (18.2)	56 (82.4)	29 (42.6)	3 (4.4)	2 (50)	2 (50)	3 (75)	2 (100)	2 (100)	2 (100)
IntI2 n (%)	2 (100)	1 (50)	1 (50)	1 (50)	3 (100)	1 (33.3)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)
IntI3 n (%)	24 (100)	20 (83.3)	20 (83.3)	7 (29.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Prevalence of integron classes associated with resistance genes

Results of stool samples showed a high prevalence (95.5%) of *E. coli* isolates that harboured both the *IntI1* and *bla* *TEM* genes. This prevalence was higher ($p < 0.0001$) than that of isolates that harboured both *IntI1* and *bla* *CTX-M* (70.5%), *bla* *OXA-1* (75%), and *bla* *SHV* (18.2%). The prevalence of isolates harbouring *IntI2*, *IntI3*, and the *bla* *TEM* gene was also higher ($p < 0.0001$) than those harbouring *IntI2* and *IntI3* with the *bla* *CTX-M*, *bla* *OXA-1*, and *bla* *SHV* genes.

For urine isolates carrying *IntI1* and the *bla* *TEM*, *bla* *CTX-M*, and *bla* *SHV* genes showed a prevalence of 82.4, 42.6, and 4.4%, respectively. These results showed that there was a significant difference in isolates harbouring *IntI1* and *bla* *TEM*, *bla* *CTX-M*, and *bla* *SHV* genes simultaneously ($p < 0.0001$). For isolates carrying *IntI2*, 100 and 33.3% prevalence was observed with *bla* *TEM* and *bla* *CTX-M* genes, respectively. For isolates from pus and blood, only isolates carrying *IntI1* harboured *bla* *TEM*, *bla* *CTX-M*, and *bla* *OXA-1* genes (Table 5).

DISCUSSION

Integrons are genetic elements that play a major role in antibiotic resistance transmission. They can carry several resistance genes at the same time. Integrons play an essential role in disseminating drug-resistance genes among bacteria isolates (Barzegar et al., 2022). The co-occurrence of these genetic elements significantly contributes to the dissemination of antibiotic resistance in Enterobacteriaceae and has been associated with specific genes conferring resistance to β-lactams, quinolones, and aminoglycosides (Tewari et al., 2022).

The results obtained in strains isolated from stool samples showed a higher prevalence of *IntI1* (89.8%) than *IntI2* (4.1%) and *IntI3* (49%). Similar results were reported by a study in Iran where the prevalence of *IntI1*(78.26%) was higher than *IntI2* (76.81%) (Kargar et al., 2014). Furthermore, the results of a study in Spain reported by Vinue et al. (2008) showed a higher prevalence of *IntI1* than *IntI2* detected in isolates from stool (Vinue et al., 2008). Otherwise, the prevalence of *IntI3* was higher than that observed in a study in Burkina Faso (Dembelé et al., 2022). Globally, these results showed that class I integrons are extremely important for the development and transmission of resistance genes in clinical *E. coli* strains. Overall, given the high prevalence of *IntI1*, it can be suggested that multidrug resistance is associated with the presence of these *IntI1*.

Regarding urine isolates, the results showed a higher prevalence of *IntI1* (50.7%) than *IntI2* (2.2%). However, *IntI3* was not found. The results of the present study are similar to those reported by a study that was done in Iran by Khoramrooz et al. (2016), where a prevalence of IntI1 of 52 and 2.5% for *IntI2* was reported. The same study reported the absence of *IntI3* in urine isolates (Khoramrooz et al., 2016).

However, the results of this study are lower than those of Zeighami et al. (2014) who reported a prevalence of 78.8 and 4.5% for *IntI1* and *IntI2*, respectively (Zeighami et al., 2014). A recent study in Iran reported the incidence of class 1 and 2 integrons was obtained in 39.9 and 14.1% of the isolates, respectively. Class 3-integron was not detected in any of the Uropathogenic *E. coli* isolates (Nasif et al., 2022; Barzegar et al., 2022). However, results of this study were contradicted by those reported by Lin et al. (2015) in which any isolates from urine carried *IntI2* and *IntI3* (Lin et al., 2015). Overall, the results showed an absence of *IntI3* in isolates from urine, pus, blood, and vaginal swabs. This suggests that *IntI3* appears to play a minor role in resistance in these *E. coli* strains (Moura et al., 2010).

The results of this study also showed the coexistence of two or even three integrons class in certain isolates. Integrons of class 1 and 3 were found simultaneously in 24 (49%) stool isolates. Etayo et al. (2018) reported the coexistence of *IntI1* and *IntI* 2 in 8% in ESBL-producing *E. coli* (Etayo et al., 2018). Rizk and El-Mahdy (2017) reported the co-existence of more than one type

of integron in 36.9% of isolates, and a prevalence of 38% was reported by Kargar et al. (2014) in a study performed in 69 multidrug-resistant (MDR) *E. coli.* Kor et al. (2013) found only one isolate carrying both integrons among clinical isolates. Odetoyin et al. (2017) reported a prevalence of 2.4% in fecal *E. coli* isolated from mother–child pairs in Nigeria. Results of the present study revealed a prevalence of 1.2% for *IntI1* and *IntI2* simultaneously in urine isolates. Previous studies have reported the simultaneous occurrence of IntI1 and *IntI2* in 3.3% (Alkhudhairy et al., 2019). Integrons, capable of integrating, expressing, and disseminating gene cassettes carrying resistance determinants, play a critical role in facilitating the multidrug resistance (MDR) phenotype in these bacteria (Sabbagh et al., 2021).

Conclusion

This study reported the existence of class 1, 2 and 3 integrons in clinical isolates of multi-resistant *E. coli* obtained from different biological samples. Thus, class 1 integrons were observed with a high percentage. The co-existence of these integrons with resistance genes in ESBL-producing strains of *E. coli* had also been demonstrated. Hence, it is necessary to set up a surveillance system in order to better control the dissemination of resistance genes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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