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Review

The '*thanato-resistome*' - The funeral industry as a potential reservoir of antibiotic resistance: Early insights and perspectives

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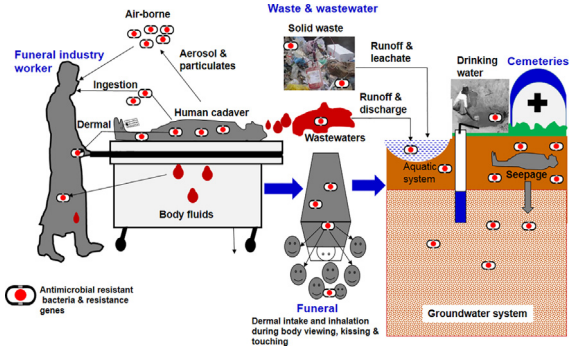
HIGHLIGHTS

- Antibiotic resistance (AR) poses significant risks to global public health.
- Mortuaries, funeral parlours and cemeteries are under-studied reservoirs of AR.
- The funeral industry and cemeteries harbour multi-drug resistant bacteria.
- Exposure risk factors, human intake pathways, and health risks are discussed.
- A mitigation framework, knowledge gaps and emerging research tools are highlighted.

GRAPHICAL ABSTRACT



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ABSTRACT

The funeral industry is a potential reservoir of antibiotic resistance. The occurrence, human exposure and health risks of antibiotic resistance in the funeral industry were examined. The funeral industry harbours antibiotic resistance to multiple common and last-resort antibiotics, hence constitutes the '*thanato-resistome*'. Hydrological processes, air-borne particulates and vectors disseminate antibiotic resistance, while horizontal gene transfer circulates antibiotic resistance among resistomes, forming a complex network. Ingestion, inhalation of air-borne particulates, dermal intake and clothes of workers contribute to human exposure. Human health risks include; development of drug resistance in previously susceptible pathogens, and increased morbidity and mortality caused by increased pathogenicity and outbreaks of multi-drug resistant infections. Ecological risks include the proliferation of resistant organisms at the expense of susceptible ones, thereby disrupting ecosystem structure and function, including biogeochemical cycles. Barring inferential data, quantitative evidence linking antibiotic resistance to human infections is weak. This reflects the lack of systematic quantitative studies, rather than the absence of such health risks. Quantitative risk assessment is constrained by lack of quantitative data on antibiotic resistance in various reservoirs and exposure routes. A framework for risk assessment and mitigation is proposed. Finally, ten hypotheses and emerging tools such as genomics, in silico techniques and big data analytics are highlighted.

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1. Introduction

Antibiotic resistance is a global issue posing human and animal health risks. Antibiotic resistance and its human health risks have received significant research attention. To date, antibiotic resistance has been detected in several bacteria, including the ESKAPE pathogens (***E**nterococcus faecium*, ***S**taphylococcus aureus*, ***K**lebsiella pneumoniae*, ***A**cinetobacter baumannii*, ***P**seudomonas aeruginosa*, and ***E**nterobacter* species), *Clostridium difficile*, *Escherichia coli* and *Proteus* (De Oliveira et al., 2020; Ma et al., 2020). The widespread use of antibiotics for human and animal health care is recognized as the key factor contributing to the development of antibiotic resistance.

Existing literature, including reviews, is dominated by studies investigating the occurrence and behaviour of antibiotic resistance in municipal and industrial wastewaters (Rizzo et al., 2013; Gwenzi et al., 2018; Smyth et al., 2020). Other studies investigated the removal of antibiotic resistance in wastewaters using various techniques (Zhou et al., 2020). The occurrence of antibiotic resistance and nosocomial infections in health care facilities such as hospitals, and veterinary facilities has also been investigated (Lien et al., 2017; Szekeres et al., 2017). By contrast, the funeral industry, including thanatopraxy care facilities and cemeteries has long been recognized as one of the least studied industries with respect to environmental pollution, including antimicrobial resistance (Ucisik and Rushbrook, 1998; Paíga and Delerue-Matos, 2016; Gwenzi et al., 2018). Yet to date, no attempts have been made to explain this trend.

The funeral industry, entailing thanatopraxy care, burial and cremation is a thriving global business. Thanatopraxy refers to the art and science of preserving human cadavers or corpses for various purposes, including medical training and burial. Thanatopraxy care serves three functions: (1) prevents the decomposition of cadavers, (2) promotes hygiene and suppresses odours, and (3) gives the cadaver, especially the face, more natural appearance to facilitate public or private viewing (Varlet et al., 2019). The funeral industry and thanatopraxy care are closely linked to health care, and play critical roles in the preparation, storage, transport and subsequent burial in cemeteries.

Research on the human health risks in the funeral industries has a relatively long history. Literature dating back to the late 1960s (Hinson, 1968), and subsequent studies conducted in the 1990s focused on the nature and occurrence of infectious diseases in funeral homes and autopsy (Nyberg et al., 2000; Healing et al., 1995; Gershon et al., 1998), while cemeteries and antibiotic resistance attracted limited attention. The early 2000s witnessed an emergence of literature on antibiotic resistance in the funeral industry specifically autopsy (e.g., Fridkin et al., 2005; Kuehnert et al., 2005). Subsequent studies, including reviews, investigated the contribution of cemeteries to inorganic and microbial pollution (Spongberg and Becks, 2000; Żychowski, 2012; Żychowski and Bryndal, 2015; Vaezihir and Mohammadi, 2016; Van Allemann et al., 2019; Silva et al., 2020). Recently, an increasing body of evidence has reported the occurrence of pharmaceuticals, antibiotic resistant bacteria and their resistance genes in environmental media from cemeteries (Carstens, 2012; Carstens et al., 2014; Fiedler et al., 2018; Abia et al., 2018, 2019). However, comprehensive reviews on antibiotic resistance in the funeral industry, including thanatopraxy care and cemeteries are still lacking. Therefore, the current review posits that, given the global presence and critical function of the funeral industry in the logistics of human cadavers, the industry is a potential global hotspot for antibiotic resistance, constituting the '*thanato-resistome*'. The term '*thanato-resistome*' refers to the complete collection of antibiotic resistant bacteria and their resistance genes in human cadavers, and environments impacted by such cadavers.

The current review summarizes the available evidence to address three hypotheses: (1) socio-cultural factors, regulatory frameworks, and researchers' attitudes and perceptions account for the limited environmental research on the funeral industry, (2) thanatopraxy care and cemeteries constitute potentila hotspot reservoirs of antibiotic resistance, (3) antibiotic resistance poses significant health risks particularly in developing countries, and (4) a risk assessment and mitigation framework, including the prevention, control and removal of antibiotic resistance can minimize the health risks. By highlighting these overlooked aspects, the purpose of current perspective is three-fold: (1) to draw public, research and policy attention to thanatopraxy care facilities and cemeteries as hotspots of antibiotic resistance, (2) to stimulate discussion and cross-disciplinary research on the topic, and (3) to bring thanatopraxy care facilities and cemeteries into mainstream environmental research.

2. Materials and methods

Scholarly databases, including Google Scholar®, Scopus®, ResearchGate®, ScienceDirect® and Clarivate's Web of Science were the key sources of literature on antibiotic resistance in thanatopraxy care facilities (e.g., mortuaries, funeral parlours, morgues) and

cemeteries. Boolean search techniques based on a combination of the key terms using 'AND'/'OR' were used to retrieve literature. Examples of search terms included, 'antibiotic resistance' and its variants; 'antibiotic resistance', 'antimicrobial resistance', 'antibiotic resistant bacteria', OR 'antibiotic resistance genes' AND 'thanatopraxy' and its variants; 'embalming', 'autopsy', and 'necropsy'. Related terms were; 'funeral industry', 'funeral service', 'funeral parlours', 'funeral homes', 'morgues', 'mortuaries', 'human cadaver(s)' and 'human corpse(s)'. Similar searches were conducted using 'cemeteries', its variants such as 'gravesite', 'graveyards', 'burial sites' and 'tombs'. Additional literature was obtained from the reference lists of the retrieved articles.

Retrieved articles were reviewed and examined for evidence related to the study objectives. In cases, where direct evidence on funeral industry, thanatopraxy care facilities and cemeteries was lacking, inferential evidence drawn from literature on human cadavers and health care facilities were then used. The current review is limited to a qualitative analysis, because available data were limited to enable a quantitative review based on bibliometric and meta-analyses.

3. The funeral industry as a potential AR hotspot

3.1. Rationale and context

Human death, and subsequent thanatopraxy care and final burial or interment in cemeteries are inevitable and common in any society. Infectious and chronic diseases caused by pathogenic microorganisms contribute to human morbidity and subsequent death. Depending on the causal pathogen, such human infections are often treated using a wide range of therapeutic drugs, including antibiotics, anti-retrovirals and antifungals (Paíga and Delerue-Matos, 2016). In cases where human death occurs, thanatopraxy care involving the use of embalming fluids then follows. Embalming fluids and other thanatopraxy care chemicals include synthetic chemicals such as antimicrobials, pharmaceuticals, personal care products, as well as disinfectants, perfumes and moisturisers and penetrating agents (Varlet et al., 2019). Human cadavers may contain residual pharmaceuticals including antibiotics used for the treatment of infectious diseases. Pharmaceuticals and antibiotics co-select for antibiotic resistance (Knapp et al., 2016; Zhao et al., 2017). Hence, solid wastes (e.g., used bandages, wipes, gloves), wastewaters and air-borne particulates from thanatopraxy care facilities may contain a complex mixture of contaminants, including pharmaceuticals, and antibiotic resistant bacteria and their resistance genes.

The discharge of solid wastes and wastewaters transfers a complex mixture of contaminants into the environmental systems (Fig. 1). Similarly, the burial and subsequent decomposition of human cadavers in cemeteries release of leachates including pharmaceuticals and microbial agents into the environment (Abia et al., 2018). First, thanatopraxy care facilities and cemeteries receive and accumulate complex mixtures of emerging contaminants and antibiotic resistance in embalming fluids, human cadavers, wastes and wastewaters. Subsequently, the contaminants, including pharmaceuticals and antimicrobial resistance genes are released into the environment via waste and wastewaters, and leachates.

3.2. Why is the funeral industry an under-studied reservoir?

To this point, it has been highlighted that, the funeral industry is a potential hotspot of emerging contaminants and antibiotic resistant bacteria. Whereas antibiotic resistance has received a lot of research attention, it is well-recognized that environmental research in the funeral industry has attracted very limited attention relative to other industries (Ucisik and Rushbrook, 1998; Fiedler et al., 2018; Paíga and Delerue-Matos, 2016). This then raises the question, '*Why has the funeral industry attracted less research attention than other industries?*' To date, limited attempts have been made to answer this pertinent question and identify the reasons behind this trend. Here, it is argued that, a combination of policy and regulatory frameworks, socio-cultural factors, and attitudes and perceptions of the research community and funders may account for this trend.

It is tempting to think that the accreditation and practice of professionals in the funeral industry is governed by regulations in the health care industry, but this is not the case in most countries. In the USA, the Bureau of Labour Statistics Standard Occupational Classification System classifies funeral service providers among commercial service providers rather than as health care professionals (Davidson and Benjamin Jr, 2006). Similarly, in Zimbabwe, the funeral industry falls under funeral assurance, which is classified under the insurance and pensions sector (Insurance and Pensions Act, 2004). Regulations governing the operations of the funeral industry are scattered in various Acts, including the Insurance Act (2004), Public Health Act (2002), Environmental Management Act (2002), and the Burials and Cremation Act (2002). The Public Health Act (2002) regulates the health industry, including infectious diseases, while the Burial and Cremation Act (2002) controls the disposal of human remains. The Environmental Management Act (2002) is responsible for regulating environmental pollution and management, including environmental impact assessments. Besides the mandatory registration with the Insurance and Pensions Commission of Zimbabwe, the accreditation of funeral industry professionals is unclear. The Health Professionals Act (2002) and the Health Professionals Authority of Zimbabwe specify mandatory registration for health institutions and professionals in the health care industry, including medical doctors, specialist doctors, nurses and technicians. The Act does not include funeral industry professionals. The classification of the funeral industry under commercial service providers implies that, regulations, and best practices in safety, health and environment procedures commonly used in the health care industry may not be mandatory in the funeral industry. In addition, people without a medical background, and with no understanding of the health risks are likely to be employed in the funeral industry. This also points to the misconception that, the funeral industry, including thanatopraxy care and cemeteries pose low health risks.

Research on antibiotic resistance in the funeral industry can be considered as lying at the boundary between medical sciences and environmental sciences. On the one hand, environmental scientists may regard antibiotic resistance and thanatopraxy care, including embalming as part of medical sciences. On the other hand, research by medical scientists is often limited to human health, including the prevention and control of human infections in clinical settings such as medical schools and hospitals. Thus to medical scientists, the disposal of solid wastes and wastewaters from thanatopraxy care facilities, and human cadavers in cemeteries falls under environmental sciences, rather than medical sciences. This notion is also reflected in the education system, where aspects related to industrial production systems (e.g., heat stress, fatigue, working at heights) tend to dominate the occupational safety, health and environment curricula relative to those pertinent to the funeral industries (e.g., infectious materials). Evidence shows that when a research subject falls between two disparate disciplines, it tends to be neglected by both disciplines (Gwenzi and Sanganyado, 2019). In other words, the subject falls in 'no man's land', thereby creating a research vacuum. This scenario has been pointed out in the case of water-borne diseases, where research on environmental drivers and reservoirs of cholera received a cursory attention relative to epidemiology (Gwenzi and Sanganyado, 2019).

In environmental sciences and other disciplines, the decision to investigate particular environmental phenomena, in this case, antibiotic resistance in the funeral industry, is based on whether such phenomena have been widely investigated in earlier studies. This is referred to as the 'Matthew' or 'bandwagon' effect, an emerging phenomenon in research (Daughton, 2014; Baveye, 2020a, 2020b). The bandwagon effect implies that trending research areas tend to be over-subscribed, while other equally relevant topics are neglected. The 'bandwagon/Matthew effect' may unduly over-estimate or inflate the significance of certain topics

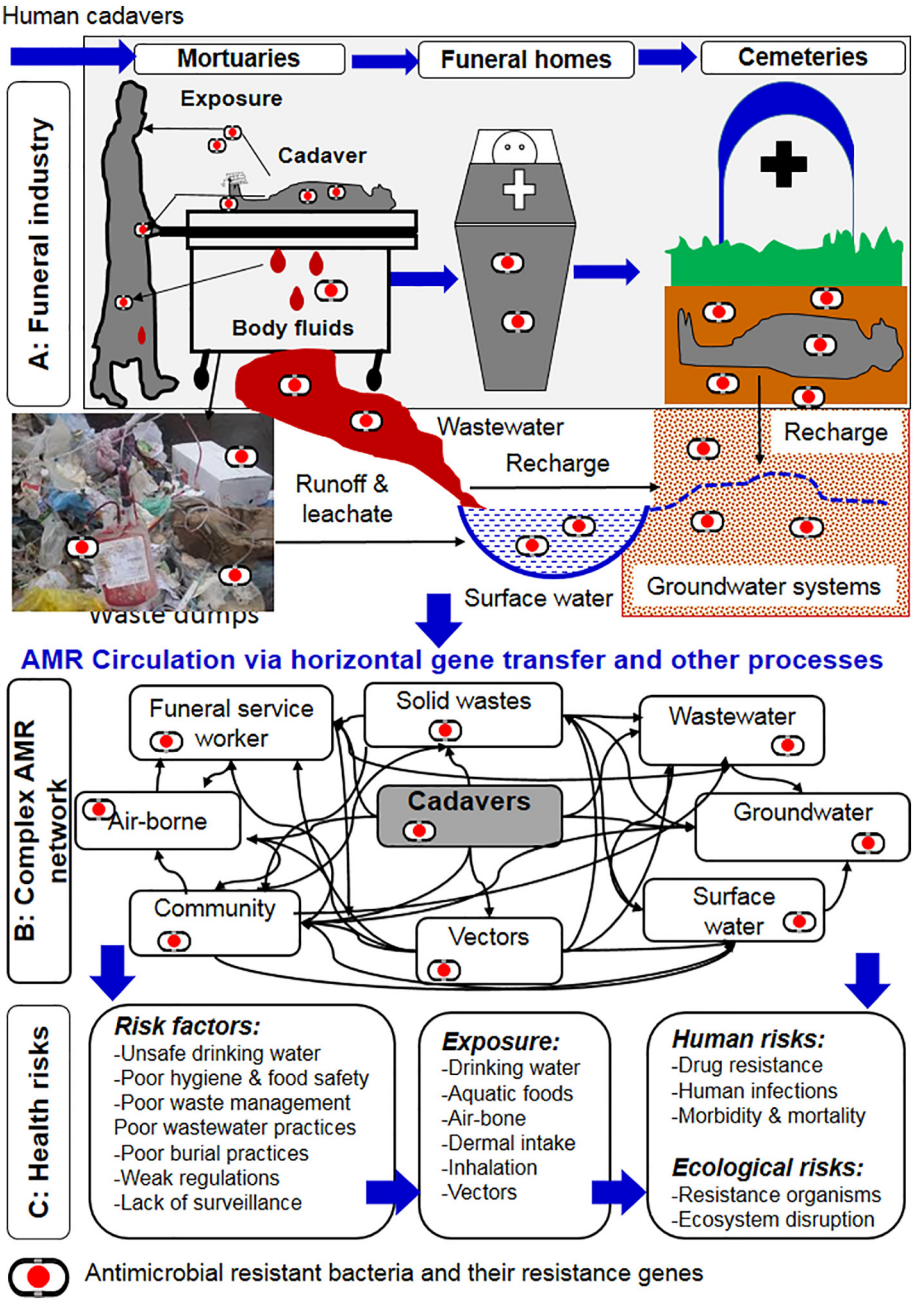

Fig. 1. Occurrence of antibiotic resistance (AR) in the funeral industry (A), circulation via horizontal gene transfer and other dissemination processes (B), and risk factors and health risks (C).

in a field, thereby creating a 'hyperbole' (Baveye, 2020a, 2020b). The potential causes, detrimental effects of the 'bandwagon' and 'hyperbole' phenomena on research, and the need to overcome them have been discussed (Baveye, 2020a, 2020b).

Others may also argue that, the lack of research attention on AR in the funeral industry reflect the lack of unequivocal evidence on the human health risks. A qualitative comparison of research on AR in wastewater and drinking water versus that in the funeral industry does not support this notion. For example, AR in wastewater and drinking water systems, and even soils has attracted significant research attention, as evidenced by several research articles, including reviews (Gwenzi et al., 2018; Sanganyado and Gwenzi, 2019). By contrast, only a handful of papers are available on AR in the whole funeral industry, spanning several compartments, including autopsy, thanatopraxy and cemeteries (Carstens, 2012; Carstens et al., 2014; Abia et al., 2018, 2019). Yet similar to AR in the funeral industry, quantitative data directly linked the occurrence of AR in wastewaters and drinking water to human health risks are scarce. This suggests that, the lack of evidence linking AR in the funeral industry cannot be the key reason for the lack of research on the topic. Rather, this lack of research on AR in the funeral industry could reflect an interplay of several factors, including socio-cultural factors.

Socio-cultural factors, and attitudes and perceptions among both researchers and funders may also explain the limited research in the funeral industry. The fear and risk of contracting pathogenic organisms and infectious diseases may also act as deterrents to environmental researchers who often lack prior training in managing the human health risks associated with human cadavers. Research on thanatopraxy care and cemeteries may not be considered as attractive, partly because, besides the funeral service professionals, most people rarely come into contact with human cadavers. Anecdotal evidence shows that, in some socio-cultural settings particularly in Africa, a strong resentment exists

with respect to dealing with human cadavers, unless one is related to the deceased person. In such socio-cultural settings, any activities, including research dealing with human cadavers (e.g., in thanatopraxy care and cemeteries) are highly stigmatized, and considered a taboo that brings bad omen, including mental health problems. The current author is no exception; this manuscript was conceived about 10 years ago, but collaborators were unwilling to contribute citing socio-cultural reasons. During the same period, no research student has expressed interest to study emerging contaminants and antibiotic resistance in wastewaters and leachates from thanatopraxy care facilities and cemeteries compared to other topics. In view of the foregoing reasons, it is not surprising that literature documenting emerging contaminants and antibiotic resistance in thanatopraxy care facilities and cemeteries remains limited. It is likely that this trend will persist in the foreseeable future.

4. The '*thanato-resistome*'

In summary, the human cadavers, autopsy, thanatopraxy and cemeteries, which form the funeral industry can be considered as resistomes or hotspot reservoirs of antibiotic resistance and their resistance genes. Collectively, these antibiotic resistance pools or reservoirs constitute the '*thanato-resistome*'. '*Thanato-resistome*' is derived from '*thanatos*', the Greek term for death, and 'resistome'. The term antibiotic resistome or simply resistome, which is widely used in literature on antibiotic resistance, refers to the complete collective assemblage of antibiotic resistant bacteria and their resistance genes in a microbial ecosystem (Ho et al., 2020). An antibiotic resistome acts as a reservoir that receives, harbours, and subsequently transfers antibiotic resistance. The term '*thanato-resistome*' is related to 'thanatomicrobiome', an emerging term increasingly used to refer to the study of post-mortem microbial communities in human internal organs and orifices after death (Can et al., 2014; Javan et al., 2016, 2017). Hence, '*thanato-resistome*' refers to the complete collection of all antibiotic resistant bacteria and their resistance genes, and the associated mobile genetic elements in a microbial ecosystem in human cadavers, and environmental compartments and media impacted by such cadavers. The '*thanato-resistome*' is conceptualized as consisting of the following: (1) human cadaver resistome, (2) autopsy resistome, (3) funeral home resistome, and (4) cemetery resistome. Like antibiotic resistance in other resistomes, the antibiotic resistant bacteria and their resistance genes in the '*thanato-resistome*' could potentially undergo continuous exchange among the resistomes, and the surrounding environment. This circulation of antibiotic resistance may occur via various processes mediated by horizontal gene transfer, anthropogenic activities and hydrological processes. Further studies are required to investigate the circulation and mechanisms of antibiotic resistance within the '*thanato resistome*', and subsequent exchange with other resistomes. Here, early insights and perspectives on the occurrence and sources of antibiotic resistance in each resistome are highlighted.

4.1. The human cadaver resistome

Human cadavers are putative hotspot reservoirs of intrinsic or induced antibiotic resistance derived from the human resistome. The human resistome consists of various sub-compartments, including the gut, oral, urogenital and skin resistomes (Pal et al., 2016; Ho et al., 2020). Evidence shows that the human resistome is a hotspot reservoirs of a broad spectrum of antibiotic resistant bacteria and their resistance genes (Pal et al., 2016; Ho et al., 2020; McInnes et al., 2020). For example, studies show that the urogenital and oral antibiotic resistomes harbour resistance genes to macrolides and tetracyclines (Pal et al., 2016). The human skin and airway antibiotic resistomes contain several resistance gene classes, including; those encoding for resistance to multiple drugs, tetracycline, macrolide, amphenicol and aminoglycoside (Pal et al., 2016).

Several studies, including reviews, have reported antibiotic resistant bacteria and their resistance genes in both healthy and unhealthy populations (Forslund et al., 2014; Ho et al., 2020; Yokoyama et al., 2020). To date, several resistance genes have been detected, including those encoding resistance to the following antibiotics, among others: (1) macrolides (*ermB*, *ereA*), (2) aminoglycosides (*strA*, *strB*), (3) β-lactamases (bla_{TEM}), (4) sulfonamides (*sul2*), (5) trimethoprim (*dfrA14*), (6) bacitracin, and (7) tetracyclines (*tetM*, *tetQ, tetW*) (Ho et al., 2020). Antibiotic resistant bacteria detected in the human gut include the ESKAPE pathogens such as *Escherichia coli*, and *Clostridium difficile* (De Oliveira et al., 2020). Accordingly, AR has been reported in faecal and body fluids specimens from both healthy and unhealthy human beings (Yokoyama et al., 2020).

The antibiotic resistant bacteria and their resistance genes persist even after human and cell death. For example, one study showed that, extracellular and intracellular *sul1*, *sul2*, t*etW* and *tetT* antibiotic resistance genes frequently located on plasmids persisted in the environment for over 20 weeks (Mao et al., 2013). Other studies show that DNA from inactivated *Enterococcus faecalis* was still detectable using polymerase chain reaction one year after cell death, while the spontaneous degradation of hydrated DNA to short fragments may occur over several thousand years (Young et al., 2007). The maximum longevity of DNA following cell death depends on spontaneous DNA decomposition caused by oxidation, hydrolysis, alkylation and UV irradiation, which in turn, depend on environmental conditions such as temperature, mechanical stress and redox conditions (Young et al., 2007). Moreover, biofilms, which occur in the human body and the environment, are well-known to promote the persistence and proliferation of ARGs by providing protection against antibiotics and other stresses (Stalder et al., 2020). Hence, antibiotic resistant bacteria and their resistance genes derived from the human resistome are putatively carried over in human cadavers, and further propagate in the environment via various mechanisms. This notion is corroborated by autopsy studies detecting antibiotic resistance in human cadavers.

4.2. The autopsy resistome

A number of autopsy studies have detected AR in specimens from human cadavers (Cohen et al., 2010; Bates et al., 2015; Lieberman et al., 2016; Burcham et al., 2019). Autopsy reports of antibiotic resistance date back to the 1950s (DeVries and Pritchard, 1955). For example, one autopsy study of 213 cases in Montreal General Hospital, Canada, investigated chloramphenicol, penicillin, chlortetracycline and oxytetracycline resistance in *Micrococcus pyogenes* (*Staphylococcus aureus*), the causative agent for nosocomial staphylococcal infections (DeVries and Pritchard, 1955). The same authors reported that, 82% of the 213 cases, equivalent to 175 cases, had penicillin-resistant staphylococci. In addition, cases which were sensitive to chloramphenicol were resistant to chlortetracycline, penicillin, and oxytetracycline (DeVries and Pritchard, 1955).

Subsequent studies conducted in other regions including Africa have also detected antibiotic resistance in various pathogens (Cohen et al., 2010; Bates et al., 2015; Lieberman et al., 2016). For example, one study investigated HIV-associated *Mycobacterium tuberculosis* in post-mortem biopsies of lung and extrapulmonary specimens in KwaZulu-Natal, South Africa (Lieberman et al., 2016). In the same study, multidrug resistance to eight antibiotics (isoniazid, rifampicin, ethambutol, streptomycin, ofloxacin, kanamycin, ethionamide, capreomycin) was detected in specimens from three subjects, while specimens from one subject were resistant to rifampicin (Lieberman et al., 2016). A year-long autopsy study based on lung specimens conducted at the University Teaching Hospital in Lusaka, Zambia detected rifampicin-resistant *Mycobacterium tuberculosis*, which is an indicative proxy of multidrug-resistance (Bates et al., 2015). An autopsy study conducted in Japan detected *Streptococcus mitis* with multidrug-resistant to penicillin,

cephalosporins, carbapenem, macrolides, and fluoroquinolone (Matsui et al., 2013).

One study conducted at a mortuary at St Luke's Hospital in Malta investigated antibiotic resistance in *Staphylococcus aureus* in human cadavers of patients that have been hospitalized for at least 24 h versus those that have not been hospitalized in the last 6 months. Results showed that 8 out of 15 (i.e., 53%) of the hospitalized human cadavers had significantly higher ($p = 0.0505$) methicillin-resistant *S. aureus* (MRSA) than the non-hospitalized group, which had 4 out of 19 (i.e., 21%). Methicillin resistance confers bacteria with resistance to cephalosporins and penicillins (Zammit et al., 2011). In the same study, MRSA isolates were also multi-drug resistant to a range of antibiotics, including; oxacillin, erythromycin, ofloxacin, kanamycin, fusidic acid, lincomycin, tobramycin, gentamicin, fosfomycin and tetracycline.

Notably, the available autopsy data documenting AR are still very limited. Hence, several human pathogens known to be highly resistant to antibiotics are still missing in the existing autopsy literature. This trend indicates that, antibiotic resistance in human cadavers attracts limited attention compared to that in patients. This is because, determining antibiotic resistance in patients is critical in choosing the appropriate combination of antibiotics for treating human infections. Thus, barring the few cases where autopsy and forensic studies are warranted, limited value could be attached to understanding antibiotic resistance profiles in human cadavers. In addition, the limited data may also point to the difficulties associated with conducting autopsy studies. These challenges include; (1) strong cultural objections in some geographical and sociocultural and religious settings, (2) a critical shortages of qualified experts to conduct such studies, and (3) under-developed and poorly equipped pathology infrastructure and services (Bates et al., 2015). However, recent studies suggest that analysis of post-mortem human microbiome or thanatomicrobiome provides some insights on the health conditions in living populations (Pechal et al., 2018).

4.3. The funeral home 'thanatopraxy' resistome

Funeral homes or thanatopraxy care facilities are potential hotspots of antibiotic resistance derived from human cadavers, and that induced through excessive use of antimicrobials. Yet very limited data exist on antibiotic resistance in funeral homes or thanatopraxy care facilities. The few studies available report extended-spectrum beta-lactams (ESBL), and multi-drug resistant hepatitis virus, human immunodeficiency virus (HIV), tuberculosis and pathogenic fungi (Demiryurek et al., 2002; Davidson and Benjamin Jr, 2006). Antibiotic resistant strains of *Salmonella*, hepatitis A, *Shigella* species, *Cryptosporidia*, *Helicobacter* and Enterobacteriaceae have also been detected in human cadavers in thanatopraxy care facilities (Davidson and Benjamin Jr, 2006; Serefhanoglu et al., 2009).

One study conducted in an anatomy laboratory determined pathogens on laboratory coats before and after the dissection of human cadavers until the gastrointestinal tract was exposed (Kabadi et al., 2013). Results showed that laboratory coats of 19 out of 67 students (i.e., 28.4%) had *S. aureus*, 8 (11.9%) had *S. pyogenes*, while 4 (6.0%) had *E. faecalis*. These values were significantly higher than those observed for *S. aureus* (13 of 67 students: 19.4%), *S. pyogenes* (5 out of 67: 7.46%) and *E. faecalis* (none) in the pre-dissection swab. However, Kabadi et al. (2013) did not test the isolates to assess their antimicrobial resistance profiles. The few available studies suggest that antibiotic resistant bacteria, including those causing human infections occur in the thanatopraxy care. However, there is a paucity of data on the occurrence and nature of antibiotic resistance in solid wastes, wastewaters and airborne particulates in thanatopraxy care facilities.

4.4. The cemetery resistome

Putrefied human cadavers can release leachate containing bacteria, viruses, and organic and inorganic contaminants, which may enter groundwater systems via seepage (Ucisik and Rushbrook, 1998). The transport of contaminants into groundwater systems via seepage depends of various factors, including geology, soil texture, depth to groundwater and existence of preferential flow pathways (Ucisik and Rushbrook, 1998). The risk of groundwater contamination is high on sandy and gravely soils with shallow groundwater systems such as wetlands. There are suggestions to consider cemeteries as a special type of landfill based on leachate chemistry (Dent and Knight, 1998; Fiedler et al., 2012). This is problematic because cemeteries are not designed and operated based on principles for engineered sanitary landfills. Specifically, cemeteries lack hydraulic liners to prevent leachate, and leachate monitoring and treatment systems.

Pharmaceuticals, antibiotics and inorganic contaminants have been detected in environmental media from cemeteries, including surface water and groundwater (Carstens, 2012; Carstens et al., 2014; Paíga and Delerue-Matos, 2016; Fiedler et al., 2018; Abia et al., 2018, 2019). Pharmaceuticals, nutrients and trace metals are well-known for inducing and co-selecting for the emergence of antibiotic resistance (Knapp et al., 2016; Zhao et al., 2017). Accordingly, antibiotic resistant bacteria and their resistance genes have been reported in environmental media from cemeteries, and a number of studies conducted in cemeteries in South Africa are quite instructive in this regard (Carstens, 2012; Carstens et al., 2014; Abia et al., 2018, 2019).

Abia et al. (2018) tested *E. coli* isolates from surface and groundwater samples from a cemetery in Cape Town, South Africa, for susceptibility to eight antibiotics using the disk diffusion method. The eight antibiotics belonging to different classes and the corresponding amounts used were: (1) Phenicol (chloramphenicol, C: 25 μg), (2) tetracycline (T): 25 μg, (3) cephalosporin (cephalexin, CFX: 30 μg), (4) folate pathway inhibitor (trimethoprim, TM: 5 μg), (5) fluoroquinolone (ciprofloxacin, CIP: 5 μg), (6) nitrofuran (nitrofurantoin, NI: 300 μg), (7) cephalosporin-lactamase inhibitor (ceftolozane/tazobactam, C/T: 40 μg), and (8) aminoglycoside (streptomycin, S: 10 μg). The results showed that 87 of the *E. coli* isolates were resistant to at least one of the eight tested antibiotics (Abia et al., 2018). The highest antibiotic resistance of 71% was observed for streptomycin and trimethoprim, while the lowest values were observed for chloramphenicol (26%) and nitrofurantoin (28%) (Fig. 2).

Abia et al. (2018) also classified those isolates that were resistant to three or more antibiotic classes as multi-drug resistant. The results showed that 86 out of 87 isolates, equivalent to about 98.9% were resistant to at least two antibiotics. Only one isolate (i.e., 1.1%) was resistant to a single antibiotic ceftolozane/tazobactam (C/T), which is a cephalosporin-lactamase inhibitor (Table 1). A total of 72 out of the 87 isolates (i.e., 82.8%) were classified as multi-drug resistant, while 22 out of 87 (i.e., 25.3%) were resistant to four different antibiotics. Table 1 shows that a total of four isolates were resistant to all eight antibiotics tested by Abia et al. (2018). The most abundant multi-drug resistant phenotypic group belonged to S-T-TM-CIP with 13 isolates, and 4 of them were from the borehole water samples (Abia et al., 2018). The remaining four resistant isolates from the borehole water samples were two each presenting S-T and S-T-TM-CIP-C/T phenotypes. In addition, 41 out of 87 isolates (i.e., 47.1%) tested positive for at least one of the virulence genes, while 36 out of 87 isolates were pathogenic. However, both studies by Abia et al. (2018, 2019) were limited to water samples from surface water bodies and boreholes in cemeteries, but did not include a control samples from outside the cemeteries. Hence, the degree of enrichment of antibiotic resistance due to cemeteries relative to background values could not be estimated.

In another study conducted on cemeteries in Cape Town and Mpumalanga (South Africa), Abia et al. (2019) investigated bacterial diversity and function in cemetery soils at the surface (0 cm) and below the depth of burial (2 m) using 6S rRNA-based metagenomic analysis. Significant differences were observed between the two depths, with one cluster of surface samples dominated by *Prauserella* and *Staphylococcus*, while *Pseudomonas* and *Rhodococcus* were dominant in the 2-

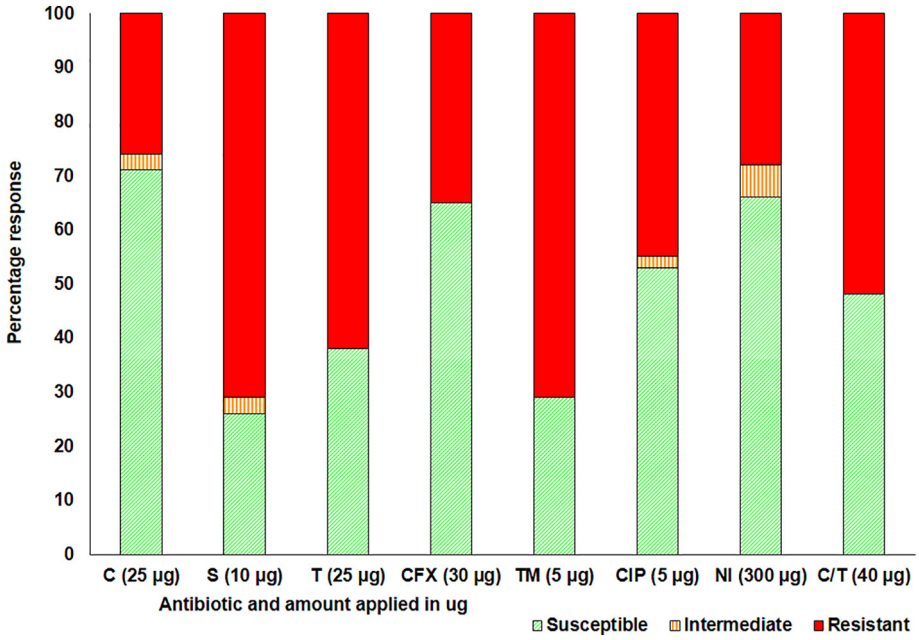

Fig. 2. Percent distribution of resistant, intermediate and susceptible *Escherichia coli* isolates in surface and groundwater samples from a cemetery in Cape Town (South Africa) with respect to resistance to eight antibiotics. C: chloramphenicol; S: streptomycin. T: Tetracycline; CFX: cephalexin; TM: trimethoprim; CIP: ciprofloxacin; NI: nitrofurantoin; C/T: ceftolozane/tazobactam. Drawn based on data in Abia et al. (2019).

m depth cluster. The 2-m depth had a lower alpha diversity, but higher abundances of human disease functional groups than surface samples (Abia et al., 2019). The human disease functional profiles were associated with the following; (1) cancers, (2) cardiovascular diseases (e.g., hypertrophic cardiomyopathy), (3) immune system diseases (e.g., systemic lupus erythematosus), (4) infectious diseases (e.g., cholera, tuberculosis), (5) metabolic diseases (e.g., Type I and II diabetes), and (6) neurodegenerative diseases (e.g., Alzheimer's disease). The infectious human disease signatures (e.g., for cholera) were the most prevalent (Abia et al., 2019).

In an independent study conducted in aquifers in two catchments in the North Western province in South Africa, six isolates of *Pseudomonas*, *Leifsonia*, *Comamonas*, and *Brevundimonas* spp. from surface and groundwater samples were tested for resistance to eight antibiotics (Carstens, 2012; Carstens et al., 2014). The eight antibiotics and their corresponding concentrations were: (1) Amoxicillin (A): 10 µg, (2) cephalothin (KF): 30 µg, (3) ciprofloxacin (CIP): 5 µg, (4) kanamycin (K), 30 µg, (5) erythromycin (E): 15 µg, (6) chloramphenicol (C): 30 µg, (7) vancomycin: 30 µg, and (8) penicillin (P): 10 µg. Results showed that the six isolates belonged to four antibiotic resistant phenotypes: (1) S-C-KF-E-A (*Pseudomonas plecoglossicida*, *Pseudomonas fluorescens*, *Comamonas* spp.), (2) KF-A (*Leifsonia xyli*, *Pseudomonas* spp.), (3) CIP-A (*Brevundimonas diminuta*), and (4) KF (*Pseudomonas stutzeri*). Evidently, barring *Pseudomonas stutzeri*, which are resistant to one antibiotic, all the other isolates were resistant to at least two antibiotics. *Pseudomonas plecoglossicida*, *Pseudomonas fluorescens* and *Comamonas* spp. were resistant to the same five antibiotics. The same authors tested antibiotic resistance in isolates from amoeba, which feed on bacteria, and observed that the amoeba harboured antibiotic resistant bacteria (Carstens, 2012; Carstens et al., 2014). Thus, the amoeba act as reservoirs of antibiotic resistant bacteria, and possibly their resistance genes, and even contribute to horizontal gene transfer of antibiotic resistance (Pagnier et al., 2013).

In a study using *Bacillus subtilis* and *E. coli* as tester strains, Muniz and Scott (2020) identified antibiotic-producing isolates in soils collected near cemeteries in Illinois. The study by Muniz and Scott (2020) did not investigate antibiotic resistance profiles and identify the specific antibiotic-producing bacterial strains. The occurrence of antibiotics points to the possible existence of antibiotic resistant bacterial strains in such soils. Moreover, several studies have detected inorganic and microbiological contaminants of anthropogenic origin in groundwater from cemeteries (Rodrigues and Pacheco, 2010; Ucisik and Rushbrook, 1998; Oliveira et al., 2013). Although these studies did not investigate antibiotic resistance, a possibility exists for the co-occurrence and co-transport of such microbial contaminants harbouring antimicrobial resistance.

Overall, existing evidence demonstrates that antimicrobial resistance occurs in environmental media impacted by cemeteries (Carstens, 2012; Carstens et al., 2014; Abia et al., 2018, 2019), suggesting that virulent antimicrobial resistant pathogens may lurk in the sub-surface in cemeteries following the burial of the human cadaver for a longer period than often expected, thereby posing human health risks for years to come. The few available studies highlighted here provide only a snapshot on antibiotic resistance in cemeteries, highlighting the need for further research.

4.5. Antibiotic resistance genes

Compared to antibiotic resistant bacteria, literature on antibiotic resistance genes in the funeral industry and cemeteries is even scarcer. An exception is the study by Abia et al. (2019), who detected antibiotic resistance genes for beta-lactams. The antibiotic resistance genes and concentrations and diversity of antibiotics were higher at 2-m samples than for surface samples. Moreover, Abia et al. (2019) observed in-situ biosynthesis of antibiotics in cemeteries, adding to the residual antibiotics in human cadavers. The antibiotics detected included; tetracyclines, clavulanic acid, vancomycin, novobiocin, butirosine and neomycin, streptomycin; penicillins and cephalosporins, ansamysin and 12-, 14- and 16-membered macrolides (Abia et al., 2019). The biosynthesis of antibiotics by soil microorganisms is a protective mechanism that allows the microorganisms to outcompete other organisms and survive longer in the environment (Abia et al., 2019). The quantities of biosynthesized antibiotics are often small, but sub-lethal and low concentrations of antibiotics induce the development of antibiotic resistance in previously susceptible organisms (Andersson and Hughes, 2012; Abia et al., 2019).

Table 1
Antibiotic resistance profiles of *Escherichia coli* isolates in surface and groundwater samples from a cemetery in Cape Town in South Africa.
(Adapted from Abia et al. (2019).)

Nature of antibiotic resistance	Number of antibiotic resistance	Number of isolates
C-S-T-CFX-TM-CIP-NI-C/T	8 (multi-drug resistant)	4
C-S-CFX-TM-CIP-NI-C/T	7 (multi-drug resistant)	1
C-S-T-CFX-TM-CIP-C/T		2
C-S-T-CFX-TM-CIP-NI		4
C-S-T-CFX-TM-NI-C/T		4
C-S-T-TM-CIP-NI-C/T		1
C-S-CFX-TM-CIP-C/T	6 (multi-drug resistant)	1
C-S-CFX-TM-NI-C/T		1
C-S-T-CFX-TM-C/T		1
C-S-T-TM-NI-C/T		1
C-T-CFX-TM-NI-C/T		1
S-T-CFX-TM-NI-C/T		2
S-T-TM-CIP-NI-C/T		1
T-CFX-TM-CIP-NI-C/T		1
C-CFX-TM-CIP-NI 1	5 (multi-drug resistant)	1
C-S-CFX-NI-C/T 1		1
C-S-T-TM-C/T 1		1
C-T-CFX-CIP-C/T 1		1
S-CFX-TM-NI-C/T 1		1
S-T-CFX-TM-CIP 2		2
S-T-CFX-TM-C/T 2		2
S-T-TM-CIP-C/T 8		8
C-CFX-TM-NI	4 (multi-drug resistant)	2
C-S-T-C/T		2
S-CFX-TM-C/T		1
S-TM-CIP-C/T		1
S-TM-CIP-NI		1
S-T-TM-C/T		1
S-T-TM-CIP		13
T-CFX-TM-CIP		1
S-CFX-C/T 1	3 (multi-drug resistant)	1
S-T-C/T 2		2
S-T-CFX 1		1
S-TM-C/T 4		4
T-TM-C/T 1		1
T-TM-CIP		3
S-C/T 3	2 (multi-drug resistant)	3
S-T 5		5
TM-C/T 1		1
TM-CIP		1
C/T	1 (single drug-resistant)	1

Antibiotics: C: chloramphenicol; S: streptomycin. T: tetracycline; CFX: cephalexin; TM: trimethoprim; CIP: ciprofloxacin; NI: nitrofurantoin; C/T: ceftolozane/tazobactam.

It is well-established that, antibiotic resistant bacteria co-exist with their corresponding resistance genes (Tenover, 2006; Gillings, 2014; Gullberg, 2014). Intuitively, one expects that, the resistance genes corresponding to the antibiotic resistant phenotypes detected in literature also exist. Based on the available evidence on cemeteries (Abia et al., 2018, 2019; Carstens, 2012; Carstens et al., 2014) and thanatopraxy care facilities (Zammit et al., 2011) the following antibiotic resistance genes may occur, among others: (1) Methicillin resistance (*mec*) genes (e.g., *mecA*), (2) Tetracycline resistance (*tet*) genes (e.g., *tet*C, *tet*A) and beta-lactamase genes (e.g., *bla*TEM and *bla*CTX-M). These inferences are corroborated by data from studies conducted in health care facilities showing the prevalence of these resistance genes (Klingenberg et al., 2001; Boyd et al., 2004; Mulvey et al., 2011). However, further research focusing on solid wastes, wastewaters, air-borne particulates and aerosols is required to substantiate these inferences.

5. Environmental behaviour and fate

5.1. Dissemination

Hydrological and wind-driven processes control the mobilization and dissemination of antibiotic resistance (Gwenzi et al., 2018; Lüneberg et al., 2018). Specifically, wastewater discharges, surface and sub-surface runoff, infiltration, leachates and groundwater recharge mobilize and disseminate antibiotic resistance from various hotspot reservoirs into other environmental compartments, including aquatic systems (Fig. 1). In addition, surface water-groundwater interactions via baseflow and interflow allow the exchange of contaminants between surface aquatic systems and groundwater systems. Aerosols, and airborne particulates such as dust have been reported to transmit antibiotic resistance (Davidson and Benjamin Jr, 2006). In soils, sediments and groundwater systems, antibiotic resistant bacteria may undergo adsorption on solid matrix (Ucisik and Rushbrook, 1998; Wang et al., 2018).

Insects, rodents, beetles and houseflies are attracted to human cadavers (Amat et al., 2016; Al-Khalifa et al., 2020). Examples of insects associated with human cadavers and harbouring and transmitting antibiotic resistant bacteria include the German cockroach (*Blattella germanica*) and houseflies (*Musca domestica*) (Graham et al., 2009; Akter et al., 2020; Al-Khalifa et al., 2020). Therefore, insects, house flies and rodents that come into contact with, and feed on human cadavers may act as reservoirs and vectors of antibiotic resistance. However, data investigating the transmission of antibiotic resistance from human cadavers to other resistomes are still limited. Moreover, the contribution of this transmission route to antibiotic resistance in humans remains poorly understood.

Literature shows that antibiotic resistant pathogens including human immune-deficiency virus (HIV) and hepatitis C virus (HPC) may persist and remain viable in human cadavers for extended periods (Demiryurek et al., 2002; Conly and Johnston, 2005). A few cases of HIV seroconversion have been reported in occupational workers after autopsy (Douceron et al., 1993; Davidson and Benjamin Jr, 2006). Seroconversion refers to the period when the immune system starts responding to HIV by producing detectable levels of antibodies. Although bacteria harbouring antibiotic resistance will ultimately die, antibiotic resistance persists and continues circulating in the various resistomes (Pinto et al., 2010; Vittecoq et al., 2016). This persistence and continuous circulation is driven by horizontal gene transfer mediated by mobile gene elements and various dissemination processes (Fig. 1).

5.2. Horizontal gene transfer

Horizontal gene transfer mediated by mobile genetic elements disseminates antibiotic resistance into various resistance pools often known as resistomes (Gillings, 2014; Gullberg, 2014). These mobile genetic elements include; transposons, plasmids, insertion sequences, integrative and conjugative elements, integrons and prophages and gene cassettes (Gillings, 2014, 2016). In horizontal gene transfer, the term mobilome is often used to refer to mobile genetic elements and their cargo resistance genes (Gillings, 2014; Gullberg, 2014). Mobilomes transfer antibiotic resistance within and between bacterial species, and even to different species (von Wintersdorff et al., 2016). The role of the mobile genetic elements in horizontal gene transfer is discussed elsewhere (Joy et al., 2013; Gillings, 2014).

Horizontal gene transfer implies that antibiotic resistance in the gut system of human cadavers (i.e., human cadaver resistome) and cemeteries can be transferred into other resistomes. These resistomes include previously susceptible microorganisms in terrestrial and aquatic ecosystems, food animals, wastewaters, vectors, ambient air, air-borne particulates and even wildlife (Fig. 1). Other processes facilitating the dissemination of antibiotic resistance include human interactions and movements, vectors and globalization (Osborn and Böltner, 2002). The continuous circulation of antibiotic resistance among various resistomes via horizontal gene transfer and other dissemination processes results in a complex network of antibiotic resistance and resistomes (Fig. 1). These complex interactions imply that the health risks associated with antibiotic resistance in the '*thanato-resistome*' are not restricted to such environments. Instead, the antibiotic resistance is widely disseminated into other resistomes, hence posing wider health

risks. Hence, addressing antibiotic resistance and its health risks calls for a 'one health' approach. The 'one health' approach is an integrative framework that considers the interactions of antibiotic resistance in the environment, livestock, wildlife and humans, and even food safety (Institute of Medicine, 2012).

5.3. Tracking antibiotic resistance

Antibiotic resistance is ubiquitous in both natural and human-impacted environmental resistomes. Further, the continuous exchange of antibiotic resistance among resistomes via horizontal gene transfer and anthropogenic processes adds another layer of complexity to its behaviour (Fig. 1). The partitioning of antibiotic resistance in the '*thanato-resistome*' among the various reservoirs covering mortuaries, funeral homes and cemeteries is a daunting task. Thus, besides literature documenting the occurrence and characterization of antibiotic resistance (Davidson and Benjamin Jr, 2006), evidence tracing antibiotic resistance to specific sources is scarce. This reflects the lack of systematic studies profiling antibiotic resistance in solid wastes, wastewaters, airborne particulates, ambient air and even various occupational groups. In fact, most of the studies, including case reports lack control groups, robust statistical experimental design, and subsequent data analysis (e.g., Nyberg et al., 2000; Johnson et al., 1997; Sterling et al., 2000; Lauzardo et al., 2001). Inferences and conclusions based on such studies could be misleading. Systematic studies based on case-control experiments are required to profile and partitioning antibiotic resistance among the various environmental compartments in thanatopraxy care. Such information is critical in the development of effective surveillance and control systems.

The lack of evidence tracking the source of antibiotic resistance is also evident in studies focusing on cemeteries (Abia et al., 2018, 2019; Carstens, 2012; Carstens et al., 2014). These studies focused on occurrence and characterization of antibiotic resistance profiles of isolates using antibiotic susceptibility tests, but the origins remain unclear. Possible sources of antibiotic resistance in cemeteries include: (1) residual antibiotic resistance in human cadavers and its subsequent dissemination via horizontal gene transfer, (2) can be directly induced by residual antibiotics in human cadavers (Paíga and Delerue-Matos, 2016; Fiedler et al., 2018) and those biosynthesized in-situ (Abia et al., 2018), and (3) induced by inorganic and organic contaminants in leachates from human cadavers such as nutrients and metals, which are well-known to co-select for antibiotic resistance (Zhao et al., 2017). Nutrients and trace metals also promote the growth and proliferation of antibiotic resistant bacteria. Further research is required to determine the exact sources of antibiotic resistance detected in environmental samples from cemeteries. Such research may entail the validation and application of emerging tools such as genomics, in silico techniques and network analysis to establish the sources and relationships among antibiotic resistances in various environmental media.

6. Human health risk assessment and controls

6.1. Human exposure pathways

Human exposure to antibiotic resistance may occur in occupational and non-occupational settings. Occupational exposure may occur via: (1) air-borne transmission of antibiotic resistant bacteria in aerosols and particulate, (2) dermal intake through needlestick, wounds, cuts and bruises, and (3) the faecal-oral route (Tumbarello et al., 2006; Davidson and Benjamin Jr, 2006). High-risk workers in the funeral industry include autopsy pathologists, medical researchers and students, assistants and cleaners, and those involved in body bagging, washing, embalming and dressing of human cadavers. Other workers at risk include funeral directors, undertakers, grave diggers, funeral attendants, gardeners and researchers (Abia et al., 2018). Data show that cemeteries contaminate surface and groundwater systems with antibiotic resistance (Carstens, 2012; Carstens et al., 2014; Abia et al., 2018, 2019). Hence, non-occupational exposure may occur via ingestion of contaminated drinking water and aquatic foods, inhalation and dermal contact.

Occupational exposure to antibiotic resistance is not limited to the funeral industry, but may also occur in health care facilities, livestock production, and wastewater industry, among others. However, comparative data on the human exposure risks in the funeral industry to that of health care system and other industries are unavailable. Such data are critical for prioritizing research, surveillance systems and mitigation measures. Thus, quantitative health risk assessments comparing occupational exposure and health risks are required to address this gap. Such information will provide direct evidence on whether the health risks in the funeral industry are higher or lower than those in other industries. Once such evidence is available, the scientific community, policy makers and the public will avoid relying on speculations and perceptions.

6.2. Human health risks

The global burden of antibiotic resistance from the funeral industry is currently not known with certainty. However, the United Nations (2019) estimate that more than 58.4 million human deaths occurred in 2019 alone, while more than 11 million human deaths occur due to parasitic and infectious diseases (WHO, 2004). The total global deaths are expected to be higher than this estimate because several human deaths in developing countries are often unreported. The number could be particularly higher during periods of global pandemics such as COVID-19. Existing global data on human deaths are not disaggregated by funeral practices, such as embalming, cremation and burial in cemeteries. However, data from the National Funeral Directors Association (NFDA, USA) show that, in the USA, approximately 2.5 million people were embalmed in 2003 (Green, 2003; Chiappelli and Chiappelli, 2008). Thus, the global antibiotic resistance burden from the funeral industry, and the corresponding number of people exposed to antibiotic resistance could be considerably higher than currently perceived. For example, globally, it is estimated that antibiotic resistance will account for 10 million deaths per year by 2050 (De Kraker et al., 2016). Thus, further research is required to provide quantitative estimates of the global burden of antibiotic from the funeral industry.

To date, no compelling quantitative evidence exists directly linking human exposure to antibiotic resistance in the '*thanato-resistome*' to specific human health risks. Moreover, no global estimates exist on health risks and antibiotic resistance acquired via occupational and non-occupational exposure in the funeral industry. However, inferential evidence shows that human exposure to antibiotic resistance may induce resistance to single and multiple drugs in human pathogens (Tenover, 2006; Gillings, 2014). Hence, the relative contribution of the various pathways to human exposure remains unclear. The human exposure and health risks associated with antibiotic resistance in the funeral industry could be particularly high in developing countries due to the following factors among others; (1) weak and poorly enforced environmental regulations, regulatory agencies lacking expertise and resources, and severe environmental pollution (K'oreje et al., 2020), and (2) over-reliance on untreated drinking water from unprotected water sources such surface water and shallow wells (Potgieter et al., 2020).

As more evidence on antibiotic resistance in the funeral industry becomes available, scope exists for comprehensive reviews focusing on specific aspects, including the occurrence, behaviour, fate, and human exposure and health risks. Such systematic reviews based on bibliometric and meta-analytic analyses could provide further insights into the human exposure and health risks, which are currently not evident given the limited evidence base.

6.3. Health risk assessment

Risk assessment is a critical first step in health risk mitigation, and it involves hazard characterization and evaluation (Gwenzi and Chaukura,

2018; US EPA, 2017). The first step seeks to identify and characterize the nature of the hazards associated with various tasks involved in thanatopraxy care and cemeteries. In the current case, this involves the determination of the nature and occurrence of antibiotic resistance along the autopsy-thanatopraxy care-cemetery pathway. In addition, the human population at risk (e.g., cleaners, assistants), exposure pathways (e.g., inhalation, dermal intake), and the amount of the various contaminants taken via the various intake routes are determined.

The second step is risk evaluation, involving a qualitative or quantitative estimation of the likelihood or probability of occurrence of a health hazard, and the human and ecological consequences of the hazard if it occurs (US EPA, 2017). In qualitative risk assessment, the likelihood of occurrence and consequences, and the overall health risk can be ranked qualitatively as 'extremely high', 'high', 'moderate' and 'low/negligible'. In quantitative risk assessment, hazard characterization data are used to calculate a risk quotient or its variants (US EPA, 2017). In both qualitative and quantitative risk assessment, the resulting overall risk is compared to a set threshold conditions which is considered to be acceptable.

A key challenge in health risk assessment for antibiotic resistance is that the minimum threshold concentrations required to trigger adverse ecological and human health risks are not yet established. A heuristic approach could entail using data for non-impacted environments (control) as the baseline or acceptable value. In cases where ecotoxicological data exist for specific types of antibiotic resistance, threshold values such as the no observable effect concentrations (NOEC) can be used. Ultimately, the resulting health risk is used to identify health risks that require mitigation, and for prioritization of allocation of resources. The overall goal of risk assessment is to ensure that the level of health risks in the funeral industry is understood so that unacceptable health risks are mitigated.

6.4. Risk mitigation and removal of antibiotic resistance

The mitigation of health risks involves identification and evaluation of appropriate mitigation measures. This takes into account the nature of the health risks, and the feasibility and cost of the mitigation interventions. In most cases, based on the severity and consequences of health risks, a mitigation strategy encompassing a combination of preventive and control methods based on 'soft' or 'hard' engineering solutions may be required to safeguard ecological and human health. Subsequent monitoring, evaluation and feedback are undertaken to improve performance. 'Soft' engineering mitigation measures include; (1) implementation of occupational health and safety procedures, and (2) raising awareness among the public and workers about the potential health risks through training and educational campaigns. At global level, as with other human infectious diseases, specialized United Nation agencies such as the World Health Organization (WHO) in collaboration with other international agencies and developed countries could assist in developing national surveillance systems. Specific assistance targeting the funeral industry and key stakeholders may include: (1) formulating national strategy on the development of policy, institutional and regulatory frameworks, (2) facilitating the development of national monitoring and control systems, (3) capacity building at national and regional levels through regular training workshops, and (4) mobilizing resources, including technical experts, funding and laboratory equipment.

'Hard' engineering solutions include the removal of antibiotic resistance in aqueous systems through the application of conventional and advanced water and wastewater treatment methods (Sanganyado and Gwenzi, 2019). Conventional water treatment methods such as filtration have limited capacity to remove antibiotic resistance, while chlorination forms carcinogenic disinfection by-products (Hiller et al., 2019; Sanganyado and Gwenzi, 2019; Smyth et al., 2020). Thus, advanced oxidation methods with high removal efficiencies are often used. Advanced oxidation methods combine ultraviolet radiation with photocatalysts and strong oxidizing agents such as $Fe^{2+}/^{3+}$, H_2O_2, TiO_2, O_3, O_3/H_2O_2, O_3/ultra-violet, H_2O_2/ultra-violet, TiO_2/ultra-violet, O_3/catalyst, O_3/H_2O_2/ultra-violet, and Fenton and photo-Fenton processes, and their combinations (Michael et al., 2020; Zhou et al., 2020). However, advanced oxidation processes have high energy and chemical requirements (Arzate et al., 2019). These water treatment processes can be used to design on-site wastewater/leachate treatment systems for thanatopraxy care facilities and cemeteries.

Compared to conventional and advanced processes commonly used in centralized water and wastewater treatment, limited data exist on the capacity of low-cost methods commonly used for water treatment in developing countries and humanitarian emergencies. These low-cost methods include boiling, solar disinfection and slow biosand filtration. Biosand filtration relies on the formation of biofilms also known as the *Schmutzdecke* to effectively remove bacteria via entrapment and adsorption on the charged sand surfaces and subsequent die-off (O'Connell et al., 2017). Further research is required to provide empirical evidence on the removal and fate of antibiotic resistant bacteria and their resistance genes using such low-cost methods. Biofilms including those in biosand filters may act as reservoirs of antibiotic resistance, and promote its proliferation and persistence (Balcázar et al., 2015; Bleich et al., 2015). Hence, further research is required to better understand the removal capacity and fate of antibiotic resistance in biofilms in biosand filters.

7. Looking ahead: future perspectives and conclusions

7.1. Future research needs

(1) *Proposition 1: Nature of antibiotic resistance*
The existing data on antibiotic resistance in the funeral industry are still very small relative to the global importance of the industry. Thus, it is posited that, '*A significant proportion of other antibiotic resistant bacteria and their resistance genes exist, but are yet to be studied.*'

(2) *Proposition 2: Antibiotic resistance hotspots*
Data on the occurrence of antibiotic resistance in solid wastes, wastewaters, air-borne particulates and ambient air in thanatopraxy care facilities are scarce. This raises the question, '*How are the various types of antibiotic resistance distributed among the various environmental compartments, and in what form?*'

(3) *Proposition 3: Behaviour and fate*
Data on the behaviour, degradation kinetics and fate of antibiotic resistance remain limited, particularly in developing countries, which experience predominantly tropical climate. This raises three questions: (i) '*What are the key biogeochemical factors that control the environmental behaviour of antibiotic resistance in environmental systems in tropical environments*', (ii) '*What is the long-term fate of antibiotic resistance in various environmental systems*', and (iii) '*How do the degradation kinetics and fate of these contaminants vary between temperate and tropical environments?*' In this regard, studies based on chronosequence of cemeteries may provide some insights into the long-term behaviour and fate of contaminants.

(4) *Proposition 4: Human exposure pathways*
To devise effective mitigation measures to reduce human exposure there is need to answer the question; (1) '*What are the relative contributions of ingestion of contaminated drinking water and foods, dermal intake and inhalation to the total human intake of antibiotic resistance in occupational and non-occupational settings?*'

(5) *Proposition 5: Vector-mediated transmission*
Studies are required to answer the questions: (i) '*Are insects, rodents and flies occurring in thanatopraxy care facilities and cemeteries hotspot reservoirs of antibiotic resistance?*', and (ii) '*To what extent do such insects, rodents and flies contribute to the*

transmission of antibiotic resistance between environmental reservoirs and humans?'

(6) *Proposition 6: Ecotoxicology*
Data on ecotoxicology of antibiotic resistance is still limited, hence, there is need to investigate the following hypothesis, '*Synergistic interactions of environmentally relevant concentrations of antibiotic resistance and other environmental stressors have more adverse health effects that the individual contaminants*'

(7) *Proposition 7: Comparative occupational exposure and health risks*
Comparative research is required to address the following questions: *(1) How do human exposure and health risks to antibiotic resistance in the funeral industry compare to that of other industries such as health care, livestock production, and wastewater systems?, and (2) To what extent do the nature and burdens of antibiotic resistance vary among occupational workers in the various industries?*

(8) *Proposition 8: Low-cost removal methods*
The capacity of low-cost methods commonly used in developing countries to remove emerging contaminants and antibiotic resistance is still poorly understood. Moreover, the role of biofilms or the 'Schmutzdecke' in biosand filters in the promotion and removal of antibiotic resistance remains unclear. Taken together, this raises two questions, (i) '*To what extent do low-cost point-of-use water treatment methods such as boiling, biosand filtration and solar disinfection remove antibiotic resistance in typical contaminated drinking water?*', and (ii) '*What is the role of biofilms in biosand filters in the proliferation and removal of antibiotic resistance, and which of one of the two processes has an overriding effect?*'

(9) *Proposition 9: Health risks in developing countries*
The prevalence of several risk factors in developing countries raises questions on the validity of the current notion that antibiotic resistance in environmental systems pose negligible to low human health risks. A systematic synthesis of global literature is required to address the following hypothesis; '*Antibiotic resistance poses significant health risks in developing countries, and the risks are considerably higher than in developed regions*.'

(10) *Proposition 10: Epidemiological evidence*
Toxicological and epidemiological evidence linking specific human health risks to antibiotic resistance is still weak. Thus, case-control epidemiological studies are required to answer the question: '*Do occupational workers in autopsy, thanatopraxy care facilities and cemeteries, and those living close to cemeteries have significantly higher prevalence and burden of antibiotic resistance and certain human health conditions than those in the control group?*'

7.2. Emerging research tools

Recent advances in tools for data acquisition, analysis and visualization present unprecedented research opportunities to better understand the behaviour, fate and health risks of antibiotic resistance. For example, recent advances in molecular techniques has led to the development of genomic tools such as meta-transcriptomics, proteomics, metagenomics and (exo)metabolomics and (Nwaiwu and Aduba, 2020; Valli et al., 2020). Omics tools are ideal for understanding complex interactions such as metabolic networks and ecosystems functions such as pathogen-host and trophic interactions (Valli et al., 2020). Moreover, metagenomics are critical for investigating several unculturable pathogens including antibiotic resistant bacteria and their metabolic functions and networks (Bodor et al., 2020; Gan et al., 2020; Perdigão et al., 2020). Other recent advances include computed X-ray tomography for non-invasive 2-D and 3-D imaging of the internal structure of complex matrices (Mehrian et al., 2020). The integration of conventional research approaches in as ecotoxicology, epidemiology and health risk assessment, and emerging tools could improve our understanding of the health risks of antibiotic resistance.

The integration of conventional and emerging tools in data acquisition will potentially generate large quantities of data on: (1) nature and concentrations of antibiotic resistant bacteria and their resistance genes in various environmental media, (2) ecotoxicology, and ecological health effects, and (3) human toxicology and epidemiology. The analysis, synthesis and visualization of such dataset using conventional statistical tools are non-trivial tasks. Big data analytical tools such as machine learning, artificial intelligence and artificial neutral networks are ideal for that purpose (Hyun et al., 2020; Kim et al., 2020). Moreover, in silico techniques or computational (eco)toxicology and epidemiology, and network analysis can be used for analysis, integration and synthesis of large dataset from various sources (Vuorinen et al., 2013; Raies and Bajic, 2016). Together, the application of these tools is critical for addressing the knowledge gaps highlighted and advancing our understanding of the health risks of antibiotic resistance.

7.3. Conclusions and outlook

The current review investigated the occurrence, behaviour and health risks associated with antimicrobial resistance in the '*thanato-resistome*' consisting of human cadavers, autopsy, thanatopraxy and cemeteries. Evidence shows that the '*thanato-resistome*' is a potential hotspot reservoir of multi-drug resistant bacteria and their resistance genes. The resistance genes conferred resistance to several common and last-resort multiple drugs, including methicillin and beta-lactams. Hydrological processes, air-borne particulates, and rodents and insects harbouring antibiotic resistance disseminate antibiotic resistance. Horizontal gene transfer mediated by mobile genetic elements promotes the persistence, and dissemination of antibiotic resistance into various resistomes, culminating into a complex network. Human exposure in occupational settings occurs via the oral route, inhalation and dermal intake via wounds and cuts in thanatopraxy care facilities and cemeteries. Non-occupational exposure occurs via ingestion of contaminated water and food, inhalation of air-borne particulates and clothes of occupational workers. However, currently, there is no compelling quantitative evidence directly linking antibiotic resistance in the '*thanato-resistome*' to specific human health effects. The lack of evidence does not necessarily indicate lack of health risks. Rather, it reflects the lack of quantitative estimation of health risks caused by the corresponding lack of quantitative data partitioning antibiotic resistance among sources and exposure pathways. Thus, based on inferential evidence, human exposure to antibiotic resistance via the various intake pathways may induce the development of antibiotic resistance against common and last-resort therapeutic drugs. This in turn, increases the pathogenicity and virulence of multi-drug resistant pathogens, leading to potential outbreaks of human infections. Potential ecological health risks include proliferation of resistant organisms at the expense of susceptible ones. This may in turn, disrupt ecosystem structure and function, including biogeochemical cycles. A framework for assessing and mitigating the health risks of antibiotic resistance in the '*thanato-resistome*' was presented. Ten hypotheses/propositions were formulated to guide future research, and opportunities offered by emerging tools such as metagenomics, in silico techniques and big data analytics were highlighted.

Declaration of competing interest

The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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