Journal of Hazardous Materials 484 (2025) 136639

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Urban waste piles are reservoirs for human pathogenic bacteria with high levels of multidrug resistance against last resort antibiotics: A comprehensive temporal and geographic field analysis

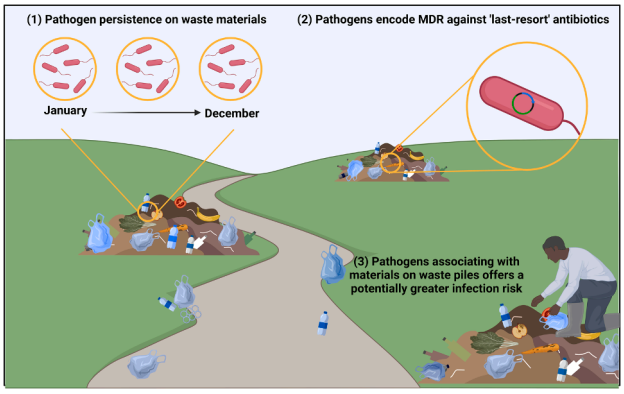
Madalitso Mphasa [a,1], Michael J. Ormsby [b,*,1], Taonga Mwapasa [c], Peter Nambala [a,g], Kondwani Chidziwisano [c,d], Tracy Morse [e], Nicholas Feasey [a,f,g,1], Richard S. Quilliam [b,1]

[a] Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Kamuzu University of Health Sciences, Blantyre, Malawi
[b] Biological and Environmental Sciences, Faculty of Natural Sciences, University of Stirling, Stirling FK9 4LA. UK
[c] Centre for Water, Sanitation, Health and Appropriate Technology Development (WASHTED), Malawi University of Business and Applied Sciences, Private Bag 303, Chichiri, Blantyre 3, Malawi
[d] Department of Public and Environmental Health, Malawi University of Business and Applied Sciences, Private Bag 303, Chichiri, Blantyre 3, Malawi
[e] Department of Civil and Environmental Engineering, University of Strathclyde, Glasgow, UK
[f] The School of Medicine, University of St. Andrews, St.Andrews KY16 9AJ, UK
[g] Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK

HIGHLIGHTS

- Potentially pathogenic bacteria are recovered at all times of the year from urban waste piles.
- Pathogen prevalence on waste materials increases before the traditional seasonally reported increase in community cases.
- Environmentally recovered bacteria encode resistance against multiple last-resort antimicrobials.
- Pathogens bound to plastic pose a heightened environmental and public health risk.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Plastisphere
Antimicrobials
Human health
LMICs
Environmental health

ABSTRACT

Inadequate waste management and poor sanitation practices in Low- and Middle-Income Countries (LMICs) leads to waste accumulation in urban and peri-urban residential areas. This increases human exposure to hazardous waste, including plastics, which can harbour pathogenic bacteria. Although lab-based studies demonstrate how plastic pollution can increase the persistence and dissemination of dangerous pathogens, empirical data on pathogen association with plastic in real-world settings are limited. We conducted a year-long spatiotemporal sampling survey in a densely populated informal settlement in Malawi, quantifying enteric bacterial pathogens

* Corresponding author.
E-mail address: Michael.ormsby1@stir.ac.uk (M.J. Ormsby).
[1] These authors contributed equally

https://doi.org/10.1016/j.jhazmat.2024.136639
Received 19 September 2024; Received in revised form 15 November 2024; Accepted 22 November 2024
Available online 26 November 2024

including ESBL-producing *E. coli*, *Klebsiella pneumoniae*, *Salmonella* spp., *Shigella* spp., and *Vibrio cholerae*. Culture-based screening and molecular approaches were used to quantify the presence of each pathogen, together with the distribution and frequency of resistance to antibiotics. Our data indicate that these pathogens commonly associate with urban waste materials. Elevated levels of these pathogens precede typical infection outbreaks, suggesting that urban waste piles may be an important source of community transmission. Notably, many pathogens displayed increased levels of AMR, including against several 'last resort' antibiotics. These findings highlight urban waste piles as potential hotspots for the dissemination of infectious diseases and AMR and underscores the need for urgent waste management interventions to mitigate public health risks.

1. Introduction

In Africa there has been a dramatic increase in plastic use, which together with poor waste management infrastructure and resources, and an inability to recycle or dispose of plastic waste adequately, has led to the continent becoming an important source of plastic pollution [1]. With improvements in the standards of living and the adoption of a 'single use' economy, Africa produces almost 18 million tonnes of plastic waste annually [2], with projections indicating that by 2060, Africa will produce an estimated 116 million tonnes of plastic waste each year [3]. Although plastics in the environment are unsightly, plastic pollution can also have substantial impacts on human health (e.g., [4-6]). Plastic is a major constituent of urban waste piles in sub-Saharan Africa, which are common in informal settlements ([7] in prep), and due to a lack of waste management facilities and incentives for complying with environmental regulations, communities living in slums and informal settlements are particularly at risk of exposure to the negative effects of waste and plastic pollution.

In urban areas, plastic pollutants, such as water bottles, sachets and bags, often block drains and sewage channels and during heavy rains can lead to localised flooding, increasing the transmission risk of waterborne diseases such as cholera, and provide breeding grounds for medically important mosquitoes [8,9]. There is now increasing evidence that plastics in the environment can also act as a reservoir for human pathogenic bacteria, viruses, and fungi [10-12]. Microbial biofilm associated with the surface of plastics, known as the plastisphere, provides protection from environmental stressors such as temperature and UV, facilitating the persistence of human pathogens and providing the potential for further dissemination [13,14]. Importantly, pathogens colonising plastic pollutants can retain, and even enhance, virulence following their association with the plastisphere, highlighting the inherent health risk associated with contaminated plastic waste in the environment [14,15].

Sub-Saharan Africa has the highest mortality rate from bacterial disease anywhere in the world, with 230 deaths per 100,000 population [16]. Enteric pathogens transmitted via the faecal-oral route, such as *Salmonella* spp., *Escherichia coli*, *Klebsiella* spp., *Shigella* spp. and *Vibrio cholerae*, are responsible for substantial numbers of these infections; and due to poor sanitation infrastructure, and high rates of open defecation, are routinely introduced into the environment. Importantly, many pathogenic bacteria now demonstrate resistance against 'last resort' antibiotics [17]. These critical drugs are used to treat severe infections caused by multidrug-resistant (MDR) bacteria when other antibiotics have failed and are often reserved for cases where conventional treatments are ineffective due to high levels of resistance. It is estimated that by 2050, almost 10 million deaths will be caused by anti-microbial resistant (AMR) bacteria, making AMR a bigger global killer than cancer [18]. Africa is disproportionately affected by the rise in AMR due to insufficient environmental health practices, poor household and healthcare infrastructure, and misuse and overuse of antibiotics, which all contribute to the transmission of AMR pathogens and a concomitant higher risk of mortality from common infections that are now resistant to standard treatments [19,20].

Rapidly increasing urbanisation in sub-Saharan Africa has increased population density and the emergence of unplanned and informal settlements in urban environments, which increases the pressure on health, environmental, and waste management resources [19,21]. Here, we have conducted a comprehensive year-long sampling survey of waste piles in a densely populated informal settlement (Ndirande) in Blantyre, Malawi, and quantified the presence of important enteric pathogens colonising the surfaces of hard plastics (e.g., PET and HDPE) and soft plastics (PE and LDPE), fabrics and organic material. Specifically, we aimed to: (1) quantify the temporal and geographic distribution of pathogenic *Salmonella* spp., *E. coli*, *Shigella* spp., *K. pneumoniae* and *V. cholerae* on waste materials in fourteen different urban waste piles; and (2) quantify the level of resistance of these pathogens to commonly used antibiotics.

2. Materials and methods

2.1. Location of sampling sites and temporal sample collection

This study was conducted in Ndirande township, an urban settlement in Blantyre, Malawi (Fig. 1). Ndirande has a population of approximately 118,000 people (15 % of the total population in Blantyre). There are no formal waste collection services in Ndirande which leads to open dumping of domestic solid waste directly into the urban environment, or on the banks of streams and rivers flowing through the settlement. Samples were collected monthly from 14 distinct waste piles (Fig. 1, Table S1) between June 2022 and July 2023. Following heavy rain, four waste piles (50, 52, 53 and 56; Fig. 1) were completely washed away, meaning samples could not be collected from each waste pile at every timepoint ([7], in prep).

2.2. Sample collection and processing

Samples of organic material (e.g., food waste and vegetation), fabric (e.g., cotton, wool, linen), hard plastics (Polyethylene terephthalate [PET] and high-density polyethylene [HDPE] [e.g., plastic bottles], subsequently called P1), and soft plastics (Low-density polyethylene [LDPE] and polyethylene [PE], e.g., plastic bags), subsequently called P2) were collected from each waste pile in triplicate from randomly allocated areas of the pile at (1) the surface and (2) 60 cm below the surface where the waste had become more compacted. Samples were placed in sterile collection bags, with a unique identifying barcode, and transported back to the laboratory for immediate processing.

Enrichment or selective culture was carried out to isolate extended-spectrum beta-lactamase (ESBL) producing *E. coli* and other ESBL-producing Enterobacteriaceae (i.e., *Klebsiella, Enterobacter* and *Citrobacter*); *Salmonella* spp.; *V. cholerae*; and *Shigella* spp. Briefly, each piece of waste material was divided into three equal sections to permit culture through three pathways, a general enrichment pathway for Enterobacterales, a pathway enriching for *Vibrio* spp. and a pathway selecting and enriching for Salmonellae. Accordingly, one section was placed into buffered peptone water (BPW) and grown overnight at 37 °C; the second section was placed into alkaline peptone water (APW) and grown for 6 h at 37 °C; and the third piece grown in bile broth (modified Enterobacteriaceae Enrichment [EE broth; Neogen] with 0.2 g/L iron pyrophosphate [Oxoid]) at 37 °C for 24 h, and then in selenite cysteine broth (Oxoid, UK) at 37 °C for a further 24 h [22].

Following enrichment in BPW, samples were plated onto ESBL CHROMagar (24 h at 37 °C) for identification of ESBL producing *E. coli* (pink colonies) and other Enterobacteriaceae (*Klebsiella/Enterobacter/Citrobacter*; blue colonies), and onto Xylose Lysine Desoxycholate (XLD) agar for putative identification of *Shigella*. Samples enriched in APW, were plated onto Thiosulphate Citrate Bile Salts Sucrose (TCBS) agar (24 h at 37 °C) to allow putative identification of *V. cholerae* (2–3 mm, flat, yellow colonies). The samples enriched in bile broth and selenite cysteine broth, were plated onto mCASE agar for putative *Salmonella* identification. These approaches will henceforth be referred to as 'culture-based screening'.

2.3. qPCR confirmation of culture-based identification

An individual colony of each isolate putatively identified by culture was picked and resuspended in 1 ml of nuclease-free water. The resultant suspension was then heated to 95 °C for 15 min to fully denature the DNA, which was subsequently used as a template for qPCR reactions and molecular-based confirmation. Strain-specific genes were targeted (primers listed in Table S2) for the identification of *K. pneumoniae* (*khe*), *Salmonella* spp. (*t tr*) *Shigella* spp. (*ipaH*) and toxigenic *V. cholerae* (*ctxA*). A second PCR was performed (*tviB*) on positively identified *Salmonella* to determine whether they were Typhoidal or non-Typhoidal strains. For amplification of *K. pneumoniae* specific genes, a Typeit 2X HRM Master mix kit (Qiagen, UK) was used; and for amplification of genes specific for *Salmonella* spp., *Shigella* spp., and *V. cholerae*, a LUNA Universal qPCR Master Mix (New England Biolabs, UK) was used. In both cases, the manufacturer's instructions were followed. qPCR was conducted in a QuantStudio 7 Flex PCR machine thermocycler. Due to confidence in the positive identification of *E. coli* on ESBL CHROMagar, qPCR confirmation was not performed.

2.4. Determination of antimicrobial susceptibility and resistance

Antimicrobial resistance testing was performed using the Kirby-Bauer disc diffusion assay. Briefly, microbank stored isolates were sub-cultured onto selective media (ESBL *E. coli* and *K. pneumoniae* on ESBL Chromagar; *Shigella* spp. on XLD; *Salmonella* spp. on mCASE; and *V. cholerae* on TCBS) and grown overnight at 37 °C. A colony of each was then further sub-cultured onto nutrient agar and grown overnight at 37 °C to obtain pure colonies. Next, pure colonies were selected from the nutrient agar, and resuspended in 5 ml sterile saline solution to obtain a turbidity of 0.5 McFarland standard. Cells were then plated on Muller-Hinton agar (Oxoid, UK). Discs (all Oxoid, UK) containing antibiotics amikacin [AK30: 30 µg]; ampicillin [AMP2: 2 µg]; co-amoxiclav [amoxicillin and clavulanic acid; AMX/CA30: 30 µg]; azithromycin [AZM15: 15 µg]; cefoxitin [FOX30: 30 µg]; ceftazidime [CAZ10: 10 µg]; cefpodoxime (CEF10: 10 µg]; ciprofloxacin [CIP5: 5 µg]; cotrimoxazole [SXT25: 25 µg]; doxycycline [DO30: 30 µg]; meropenem [MEM10: 10 µg]; pefloxacin [PEF5: 5 µg]; or tetracycline [TET30: 30 µg] were placed onto the agar using a multidisc dispenser, and the plates incubated at 37 °C for 24 h. Inhibition zones surrounding the discs were then measured, and strains were categorised as 'resistant', 'intermediate resistance', or 'sensitive' based on zones of inhibition (Table S3).

2.5. Statistical methods

All statistical analyses were conducted using Prism Software (Version 10.3.2, GraphPad). *P* values ≤ 0.05 were considered significant. To determine differences in the temporal and geographic distribution of pathogens putatively identified by the culture-based screen and confirmed by molecular methods, a mixed-effects analysis with Tukey multiple comparisons post-hoc test was performed. To determine differences between pathogens putatively identified by culture-based screen and confirmed by molecular methods, a two-way ANOVA with Holm-Šídák multiple comparisons post-hoc test was performed.

3. Results

Between June 2022 and July 2023, samples of organic material ($n = 128$ surface; 140 compacted), fabric ($n = 129$ surface; 140 compact), hard plastics (P1; $n = 130$ surface; 139 compact), and soft plastics (P2; $n = 130$ surface; 139 compact) were collected from 14 distinct waste piles in Ndirande, Blantyre (Table S4), to examine the temporal and geographic distribution of major enteric bacterial pathogens. The sampling period covered three distinct seasons, defined as the 'cold and dry season' between May and August; the 'hot and dry season' between September and November; and the 'rainy season' between

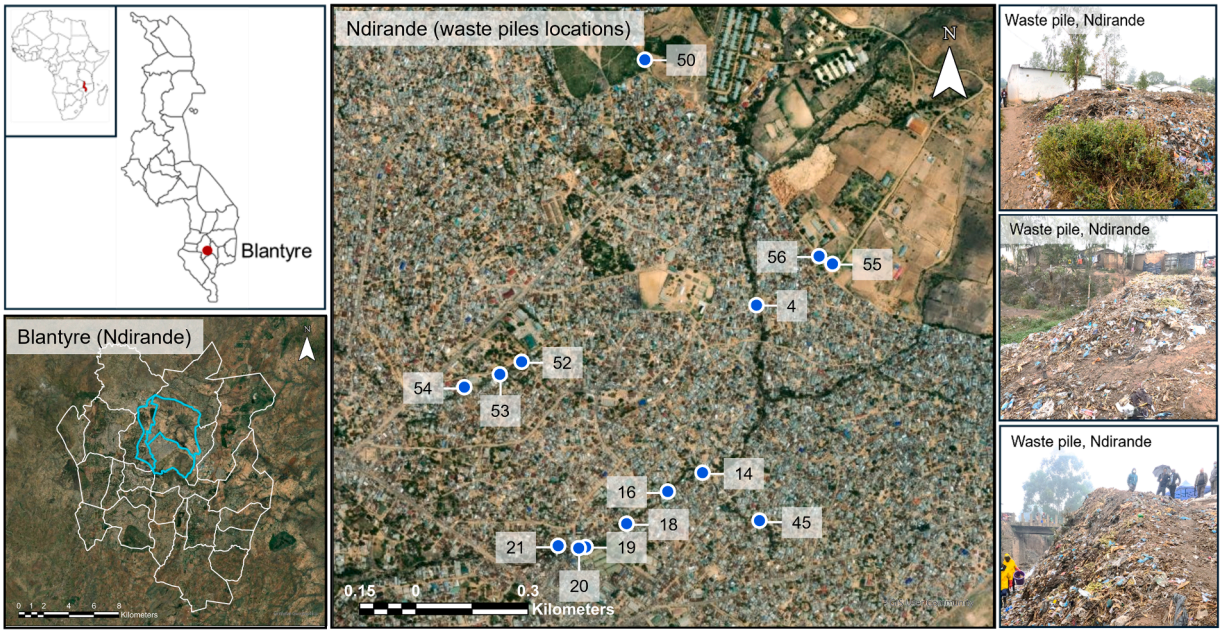


Fig. 1. : Location of urban waste piles. Fabric, organic material, and plastics were collected from urban waste piles (blue dots) in Ndirande urban settlement, Blantyre, Malawi between June 2022 and July 2023. Each distinct waste pile was located by GPS (coordinates given in Table S1).

December and April (Fig. S1).

3.1. Temporal and spatial distribution of pathogens in waste piles

Through culture-based screening, no significant differences were observed in the recovery of putative *Salmonella* spp., *Shigella* spp., or *V. cholerae* isolates between the rainy (*Salmonella* spp: 26; *Shigella* spp.: 45; *V. cholerae*: 192), cold and dry (*Salmonella* spp: 39; *Shigella* spp.: 41; *V. cholerae*: 127), or hot and dry seasons (*Salmonella* spp: 25; *Shigella* spp.: 37; *V. cholerae*: 147) (Fig. 2; Fig. S2). However, the recovery of putatively identified *V. cholerae* isolates was greater during the rainy season, and warmer periods. Significantly more ($P < 0.05$) *E. coli* and other Enterobacteriaceae (*Klebsiella*, *Citrobacter,* and *Enterobacter*) were recovered during the hot and dry season than the rainy season. Confirmatory PCR analysis supported the culture-based screen observations, with no significant differences observed in the identification of *Salmonella* spp., *Shigella* spp., or *V. cholerae* between any of the three defined seasons (Fig. 2; Fig. S3). PCR confirmation indicated that recovery of *K. pneumoniae* was significantly higher ($P < 0.05$) during the hot and dry season (97 isolates) than the rainy season (50 isolates), suggesting that the Enterobacteriaceae culture screen is proportionally representative of *K. pneumoniae*. Molecular examination revealed that all confirmed *Salmonella* spp. isolates were non-typhoidal.

ESBL *E. coli*, *Salmonella* spp., *Shigella* spp., *V. cholerae,* and ESBL Enterobacteriaceae were recovered at every waste pile examined (Fig. S2). However, the culture-based screening approach demonstrated no correlation between any of the target pathogens and the waste pile location, indicating no obvious spatial pattern within the settlement. Due to extreme weather events during the rainy season, waste piles 50, 52, 53, 54, 55, and 56 were completely washed away in December 2022, meaning that there is an incomplete temporal dataset for these waste piles.

Culture-based screening approaches putatively identified more target pathogens than via PCR (Table S5; Fig. S4); however, molecular confirmation gives a more detailed and confident identification. PCR confirmation of *Salmonella* spp. isolates suggested a degree of geographic distribution, with all isolates recovered from waste piles 55 and 56, however as several waste piles, including waste pile 56 were washed away, the dataset is incomplete. The number of positively identified isolates of *Shigella* spp., and toxigenic *V. cholerae* was too small to confidently correlate spatial distribution. PCR of candidate *K. pneumoniae* isolates confirmed they was present at each waste pile, although with no clear geographic distribution. Molecular confirmation was not conducted for ESBL *E. coli* due to the stringency and confidence in the culture-based screen for this pathogen based on genomic studies of pink isolates from ESBL-chrome in this setting [23]. There were no significant differences in the numbers of target pathogens isolated from the different types of material (Fig. S5).

3.2. Antimicrobial resistance profiles of waste-pile-associated pathogens

All isolates confirmed by PCR were screened against clinically relevant antimicrobials. In all cases, there were no significant differences in resistance profiles of isolates recovered from different waste materials, nor depending on whether the material came from the surface or the compacted portion of the waste pile (Fig. 3). Most *Salmonella* (6/14), *Shigella* (9/14), and *V. cholerae* (2/3) isolates were sensitive to all antibiotics tested. Resistance was observed in *Salmonella* isolates against cefoxitin (5/14) and pefloxacin (2/14); in *Shigella* isolates against cefoxitin (8/14), cefpodoxime (9/14), ampicillin (5/14) and cotrimoxazole (10/14); and in *V. cholerae* isolates against amoxicillin (1/3), ampicillin (1/3), doxycycline (1/3) and tetracycline (1/3). Out of the 346 *E. coli* isolates, 177 (51 %) were resistant, or showed intermediate (64; 18.4 %) levels of resistance, against at least one antibiotic. Ninety-six percent of all ESBL *E. coli* isolates were resistant to pefloxacin, and 83.8 % resistant to cotrimoxazole. One hundred percent of all ESBL *E. coli* isolates were resistant (70.2 %) or showed intermediate resistance (29.8 %) against ceftazidime. Out of the 242 *K. pneumoniae* isolates, 150 (62 %) were resistant or showed intermediate (50; 20.8 %) levels of resistance against at least one antibiotic. Almost 98 % of all *K. pneumoniae* isolates were resistant to pefloxacin, and 98.7 % resistant to cotrimoxazole. *K. pneumoniae* isolates also showed high levels of resistance against ceftazidime (69.8 %); cefoxitin (48.7 %); and meropenem (35.9 %).

4. Discussion

Urban waste piles in informal settlements can represent a considerable reservoir for pathogenic and multidrug-resistant (MDR) human pathogenic bacteria. Potentially dangerous bacterial pathogens were identified on all materials (including organic material, fabrics, and plastics) recovered from waste piles, and at all times of the year, increasing the likelihood of interaction between humans and pathogens and the subsequent human health risk. Importantly, many of the pathogens recovered from these waste piles showed resistance against several of the so-called 'last resort' antibiotics (i.e. *Klebsiella* to meropenem). Taken together, this study emphasises the considerable hazards associated with environmental waste and accentuates the urgent need for mitigation and intervention strategies directed towards plastic and other waste materials, particularly in low- and middle-income countries (LMICs) such as Africa.

In LMICs, incidences of infection by pathogenic bacteria often correlate with warmer temperatures and periods of heavy rain, which provide conducive growth conditions for bacterial enteric pathogens in the environment with greater opportunities for dissemination through irrigation, run-off, and flooding [24,25]. In Malawi, where the warmest daily temperatures are in September and October, and the heaviest rains fall between November and April [26], infections caused by *Shigella*, *E. coli*, *Salmonella*, and *V. cholerae* are most frequent ([19,27-29]). While *Klebsiella* is responsible for many infections in Malawi, particularly associated with healthcare settings [30,31], there is limited temporal data available indicating prevalence correlating with a particular season. In other global settings however, infections caused by *Klebsiella* are often more frequent during warmer and more humid periods [32,33]. Recently, it has been shown that pathogens including ESBL *E. coli* and *K. pneumoniae* frequently contaminate drains, standing water and soil, and that their abundance correlates with increased urbanisation and rainfall [34]. Our study has demonstrated that in informal settlements, the materials commonly found in waste piles are also frequently contaminated with these pathogens and clearly represent a potential exposure route for people living in close proximity to them. This risk is particularly heightened for informal waste pickers who regularly come into direct contact with these waste piles, as well as for residents using nearby rivers for domestic purposes, where waste accumulation along the banks increases the likelihood of exposure to harmful pathogens [35].

ESBL *E. coli* and *K. pneumoniae* were recovered most frequently from waste piles during the hot and dry season (Sept-Nov). Seasonality can influence the levels of infection with ESBL *E. coli* and *K. pneumoniae* in the population, with a decrease in infection prevalence during the colder season followed by an increase during the warmer, rainier months [36, 37] However, our data suggests that the increase in abundance of ESBL *E. coli* and *K. pneumoniae* in the environment likely precedes infections in the community, with continued environmental persistence observed throughout the typical infectious 'season'. This implies that as environmental conditions become warmer and more favourable for bacterial growth, isolates of ESBL *E. coli* and *K. pneumoniae* associating with waste materials in the environment, transition from biofilm-associated, dormant lifestyles, to more infectious, transmissible lifestyles.

Higher temperatures enhance metabolic processes in bacteria, with enzymes involved in degrading the extracellular biofilm matrix and those necessary for cell division and metabolism becoming more active,

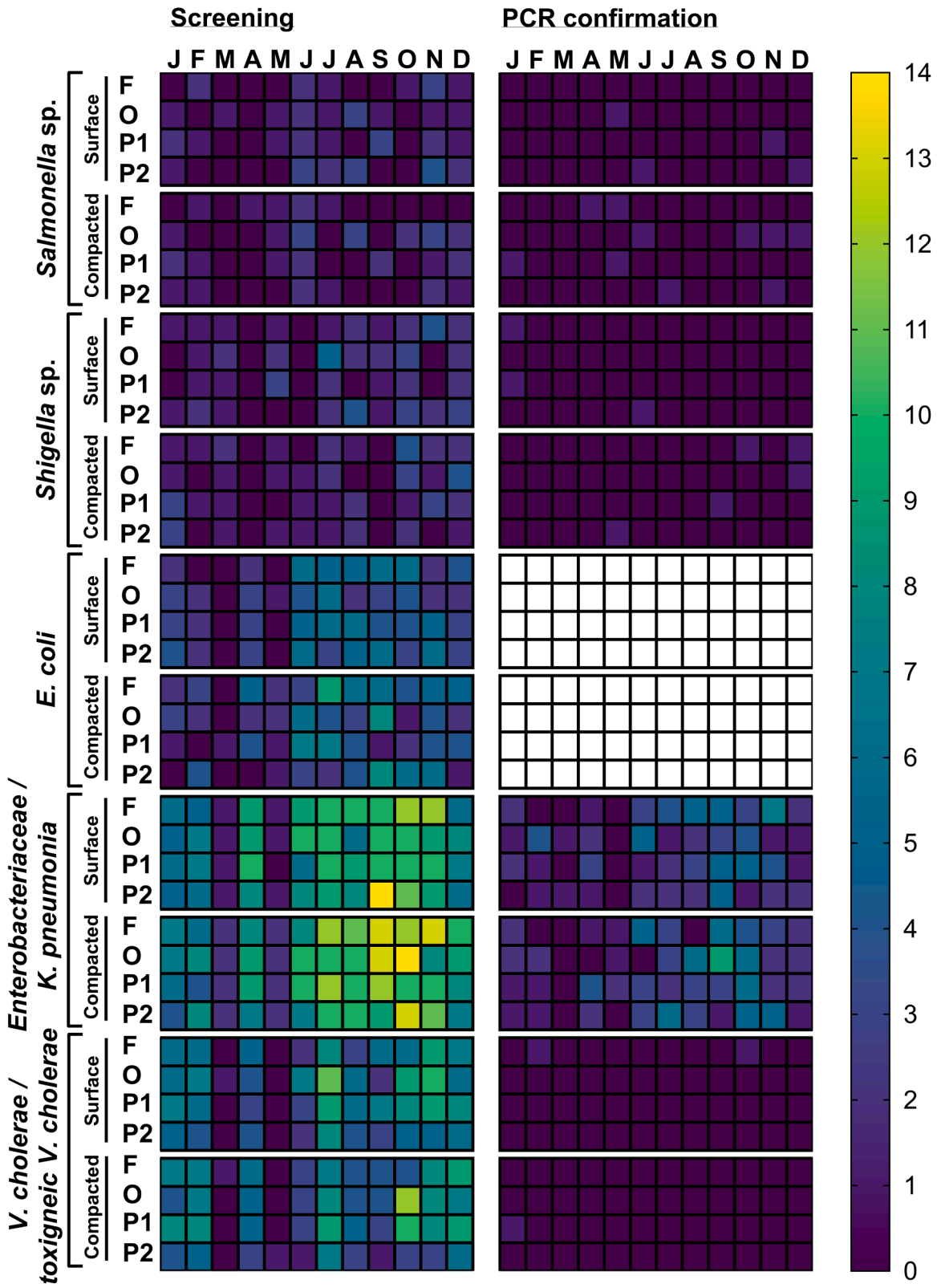


Fig. 2. : Culture-based screening and PCR confirmation of target pathogenic bacteria in 14 urban dumpsites over one year. Each square in the heat map is representative of the combined number of isolates recovered from samples of either fabric (F); organic material (O); hard plastics (P1), (e.g., PET and HDPE); and soft plastics (P2) (e.g., PE and LDPE) from 14 different waste piles. PCR of "Other Enterobacteriaceae" was used to confirm whether blue colonies growing on ESBL-chrome were ESBL *K. pneumoniae*; and PCR confirmation of *V. cholerae* isolates was confirmatory of toxigenic *V. cholerae*. *E. coli* isolates were confirmed by culture-based methods only.

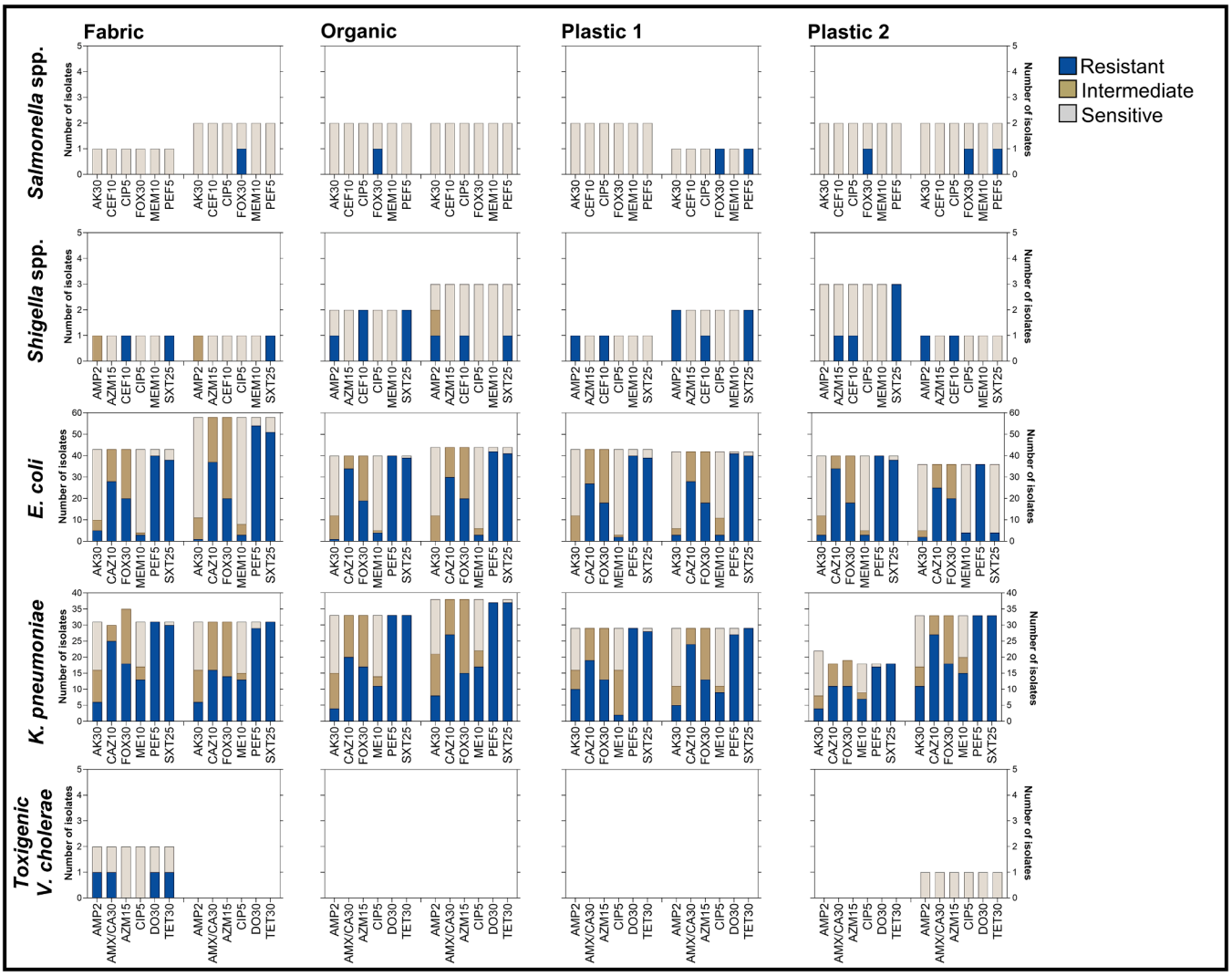


Fig. 3. AMR profiles of bacterial isolates recovered from urban waste piles. Sensitivity, intermediate resistance, and resistance were calculated using a Kirby-Bauer disc diffusion assay on isolates positively identified by PCR. Susceptibility was examined against amikacin [AK30: 30 μg]; ampicillin [AMP2: 2 μg]; coamoxiclav [amoxicillin and clavulanic acid; AMX/CA30: 30 μg]; azithromycin [AZM15: 15 μg]; cefoxitin [FOX30: 30 μg]; ceftazidime [CAZ10: 10 μg]; ciprofloxacin [CIP5: 5 μg]; cefpodoxime (CEF10: 10 μg]; cotrimoxazole [SXT25: 25 μg]; doxycycline [DO30: 30 μg]; meropenem [MEM10: 10 μg]; pefloxacin [PEF5: 5 μg]; or tetracycline [TET30: 30 μg] and resistance determined according to inhibition zones as detailed in Table S3. Note different scales on the y axes.

allowing bacteria to resume active division and growth [38,39]. Environmental factors such as increasing temperature [40], can promote biofilm dissociation and dispersal in several bacterial species, together with a concomitant upregulation of virulence genes indicating that biofilm dispersal can be a critical step for increasing bacterial virulence [41,42]. The abundance of ESBL *E. coli* and *K. pneumoniae* recovered from materials in waste piles increased in the periods preceding the traditional infectious period, suggesting that an increase in temperature could be a critical step in the resuscitation of these pathogens before the increased rainfall promotes their dissemination into the environment. Therefore, continuous environmental monitoring of pathogens (e.g., in urban waste piles) could be used as a surveillance tool to predict likely increases in community infections.

The low abundance of *Salmonella* spp., *Shigella* spp., and toxigenic *V. cholerae* found was possibly due to an inability to associate with the examined materials, however, in vitro studies have indicated that this is unlikely [13,15]. The lack of recovery was likely influenced by some species entering a viable but non-culturable (VBNC) state in the environment. The VBNC state allows bacteria to remain alive, metabolically active, and even able to acquire and spread genetic material; however, they cannot be cultured using standard laboratory techniques [43]. This allows bacteria to survive adverse environmental conditions including temperature extremes, nutrient limitations, and exposure to chemicals while waiting for more favourable conditions for proliferation. *E. coli*, *Salmonella* spp., *Shigella* spp., and *Klebsiella* spp. are all capable of entering a VBNC state in environmental settings [44-47]; however, the most well-studied organism in this context, is *V. cholerae*. Research has indicated that *V. cholerae* can transition to a VBNC state on plastics under simulated environmental conditions, before resuscitation to infectious levels [15]. Therefore, the culture-based screening approach in this study likely underestimated the abundance of these pathogens in the environment. Malawi has recently experienced one of its worst cholera outbreaks, with over 59,000 reported cases and approximately 1770 deaths as of January 2024, and isolation of the aetiologic agent from plastic waste piles is of considerable concern [48].

Enteric pathogens are associated with poor sanitation infrastructure and waste management, and a lack of healthcare provision, all of which are characteristic of informal settlements, and are further amplified in densely populated areas. Our study site encompassed 14 distinct urban waste piles, all contained within a single densely populated informal settlement (Ndirande) along a single river (the Nasolo), with many different households contributing to the same waste pile. The intestinal

microbiome of communities living in close proximity to each other is shaped by a combination of dietary practices, environmental exposures, social behaviours, and genetic factors, which contribute to a shared microbiome [49]. Consequently, it is likely that the composition of intestinal microflora, together with associated pathogen carriage and shedding, is widely shared and spread within specific communities in Ndirande, a process that could be further exacerbated by exposure to shared waste piles and this warrants further investigation.

While no significant differences were observed in the association of each pathogen with different material types in this study, the association of pathogens with plastic waste has important implications for pathogen survival, dissemination, and for human health. Plastics are durable and highly recalcitrant materials, able to withstand degradation from environmental factors including sunlight, water, and biological processes [50]. While organic materials can persist for up to six weeks and fabrics for up to five months, soft plastic polymers, such as LDPE, can persist for up to 20 years, while hard plastic polymers such as HDPE, can persist for considerably longer [50,51]. The lightweight and buoyant properties of plastics compared to organic material and fabrics, increases the potential for environmental dissemination of pathogens colonising the surface of plastics. This was particularly highlighted when extreme weather events (e.g., cyclone 'Freddy' in March 2023) disrupted and dispersed several of the waste piles in this study, with wide dissemination of the waste pile components around the settlement and to downstream receptors. In informal settlements, enteric pathogens, which are primarily faecal-orally transmitted, frequently enter urban waste piles through open defaecation and via soiled single-use diapers, which are becoming more common in LMICs [52,53]. The subsequent mixing of human faeces with waste materials provides opportunity for interactions between enteric bacterial pathogens and materials, which once on the surfaces of plastics can persist and retain their virulence even after periods of environmental stress such as desiccation, high and low temperatures, and ultraviolet (UV) radiation [11,15,54].

It is common for wild and domestic animals, e.g., flies, rodents, birds, and dogs, to interact with waste piles in urban settlements [4], which increases the opportunity for human pathogens to be transported by animals but also for animal faeces to further contaminate waste piles. In turn, this heightens the potential for wider dissemination within the community, with the additional infection risk from the spread of zoonotic pathogens [55]. Human pathogens in waste piles will encounter fluctuations in temperature and pH, which may facilitate the rapid adaption to the animal digestive tract and increase the likelihood of the evolution of novel zoonoses [56]. Future work needs to examine both veterinary and zoonotic pathogens on urban waste piles, to more fully ascertain the risk of waste piles acting as hotspots for pathogen emergence and dispersal. While this study has focused on major enteric bacterial pathogens, there are many other bacterial, viral, and eukaryotic pathogens responsible for infections in sub-Saharan Africa, although how these pathogens interact with waste materials, including plastics, is still not clear [57,58].

Although our broader culture-based screening approach commonly revealed putative isolates of *Salmonella* spp., *Shigella* spp., *V. cholerae*, ESBL *E. coli*, and ESBL Enterobacteriaceae (*Klebsiella*, *Citrobacter* or *Enterobacter*) colonising waste materials in urban waste piles, more targeted PCR indicated that only a small portion of the *V. cholerae* isolates were toxigenic (encoding cholera toxin gene *ctx*), and that all the *Salmonella* spp. were non-Typhoidal. However, many of these "non-pathogenic" isolates may also contain AMR genes with the potential for acquisition by pathogenic variants. Biofilms, including the plastisphere, are recognised hotspots for the exchange of genetic material and horizontal gene transfer (HGT) [59]. Biofilm offers greater opportunities for inter- and intra-species interaction, with a high frequency of plasmid transfer between bacteria in the plastisphere [60]. Biofilms on plastic debris can also concentrate antibiotics, which can further promote the development of AMR bacteria [61,62]. Importantly, all ESBL *E. coli* isolates recovered from materials in waste piles were resistant or showed intermediate resistance against the so-called 'last resort' antibiotic ceftazidime, and large numbers of *K. pneumoniae* isolates showed high levels of resistance against ceftazidime (69.8%) and meropenem (35.9%). Meropenem resistance is being observed with increasing frequency globally and is of major clinical concern in healthcare settings [63].

5. Conclusions

Urban waste piles are a reservoir of potentially pathogenic bacteria, and waste materials such as plastic, can pose a threat to public health by providing protection from harsh environmental conditions and facilitating pathogen replication and dissemination. Enteric human pathogens were associated with urban waste piles throughout the year, which increases the opportunities for people to be exposed to them. Importantly, many of these pathogens encode multi-drug resistance against last-resort antibiotics, which has serious ramifications for human health in the wider context of global AMR. The lack of waste management systems highlights the disproportionate burden faced by communities living in informal settlements. The potential for urban waste piles to act as hotspots for disseminating potentially pathogenic organisms further reinforces the urgent need for mitigation and intervention strategies to tackle environmental waste and the associated human health risks. This underscores the principles of One Health, emphasising the interconnectedness of human, animal, and environmental health, where unmanaged waste threatens not only human populations but also the broader ecosystem, potentially leading to zoonotic disease transmission and further exacerbating global health challenges.

Environmental implications

Plastic waste could play a critical, but as yet unrecognised, role in both the spread of infectious diseases and the amplification of antimicrobial resistance in urban environments. The persistence and distribution of plastic contamination increases the risk of direct human exposure. This study provides novel, real-world data on the association with and persistence of multiple enteric bacterial pathogens associated with plastics and other urban waste materials. These results have global significance, as plastic pollution becomes more pervasive, and climate change is altering the behaviour of enteric pathogens in the environment. This highlights an urgent need for public health interventions targeting waste management and sanitation in LMICs, with a focus on reducing plastic waste to mitigate associated health risks. Comprehensive management strategies could greatly reduce the burden of disease and curb the spread of AMR.

Funding

This work was supported by the UKRI Natural Environment Research Council (NERC) as part of the GCRF SPACES project [grant number NE/V005847/1] and the NERC Plastic Vectors project, "Microbial hitchhikers of marine plastics: the survival, persistence & ecology of microbial communities in the 'Plastisphere'" [grant number NE/S005196/1].

Ethical statement

A waiver for the work in the study was granted by the Kamuzu University of Health Sciences College of Medicine Research Ethics Committee (COMREC P.07/20/3089). Permission to sample the environment was also obtained from the Blantyre City Council who are responsible for waste management in Blantyre City including the study area. Community engagement in conducting the research was undertaken with community leaders through the Group Village Headmen (GVH).

CRediT authorship contribution statement

Nicholas Feasey: Writing – review & editing, Supervision, Methodology, Conceptualization. **Tracy Morse:** Writing – review & editing, Supervision. **Richard S. Quilliam:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Peter Nambala:** Writing – review & editing, Supervision. **Taonga Mwapasa:** Writing – review & editing, Methodology. **Kondwani Chidziwisano:** Writing – review & editing, Methodology. **Madalitso Mphasa:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Michael J. Ormsby:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

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

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.136639.

Data availability

Data will be made available on request.

References

[1] Shomuyiwa, D.O., Onukansi, F.O., Ivanova, M., Lucero-Prisno, D.E., 2023. The plastic treaty: what is in it for Africa? Public Health Chall 2 (2). https://doi.org/10.1002/puh2.83.

[2] Sadan, Z. and De Kock, L. 2022. Plastic Pollution in Africa: Identifying policy gaps and opportunities. Cape Town, South Africa.

[3] OECD. 2022. Global Plastics Outlook: Plastics use projections to 2060. Paris: OECD. doi: 10.1787/aa1edf33-en.

[4] Krystosik, A., Njoroge, G., Odhiambo, L., Forsyth, J.E., Mutuku, F., LaBeaud, A.D., 2020. Solid wastes provide breeding sites, burrows, and food for biological disease vectors, and urban zoonotic reservoirs: a call to action for solutions-based research. Front Public Health 7. https://doi.org/10.3389/fpubh.2019.00405.

[5] Wu, D., et al., 2021. Commodity plastic burning as a source of inhaled toxic aerosols. J Hazard Mater 416, 125820. https://doi.org/10.1016/j.jhazmat.2021.125820.

[6] Wang, X., Firouzkouhi, H., Chow, J.C., Watson, J.G., Carter, W., De Vos, 2023. Characterization of gas and particle emissions from open burning of household solid waste from South Africa. Atmospheric Chemistry and Physics 23 (15), 8921–8937. https://doi.org/10.5194/acp-23-8921-2023.

[7] Mwapasa, T., et al., 2024. Key environmental exposure pathways to antimicrobial resistant bacteria in southern Malawi: a saniPath approach. Sci Total Environ 945, 174142. https://doi.org/10.1016/j.scitotenv.2024.174142.

[8] Maquart, P.-O., Froehlich, Y., Boyer, S., 2022. Plastic pollution and infectious diseases. Lancet Planet Health 6 (10), e842–e845. https://doi.org/10.1016/S2542-5196(22)00198-X.

[9] Stoler, J., 2017. From curiosity to commodity: a review of the evolution of sachet drinking water in West Africa. WIREs Water 4 (3). https://doi.org/10.1002/wat2.1206.

[10] Gkoutselis, G., Rohrbach, S., Harjes, J., Obst, M., Brachmann, A., Horn, M.A., et al., 2021. Microplastics accumulate fungal pathogens in terrestrial ecosystems. Sci Rep 11 (1), 13214. Available at: /pmc/articles/PMC8282651/ [Accessed: 26 July 2022].

[11] Metcalf, R., White, H.L., Moresco, V., Ormsby, M.J., Oliver, D.M., Quilliam, R.S., 2022. Sewage-associated plastic waste washed up on beaches can act as a reservoir for faecal bacteria, potential human pathogens, and genes for antimicrobial resistance. Mar Pollut Bull 180, 113766. https://doi.org/10.1016/J.MARPOLBUL.2022.113766.

[12] Moresco, V., Charatzidou, A., Oliver, D.M., Weidmann, M., Matallana-Surget, S., Quilliam, R.S., 2022. Binding, recovery, and infectiousness of enveloped and non-enveloped viruses associated with plastic pollution in surface water. Environ Pollut 308, 119594. https://doi.org/10.1016/J.ENVPOL.2022.119594.

[13] Ormsby, M.J., White, H.L., Metcalf, R., Oliver, D.M., Feasey, N.A., Quilliam, R.S., 2024. Enduring pathogenicity of African strains of Salmonella on plastics and glass in simulated peri-urban environmental waste piles. J Hazard Mater 461. https://doi.org/10.1016/j.jhazmat.2023.132439.

[14] Ormsby, M.J., Woodford, L., White, H.L., Fellows, R., Quilliam, R.S., 2024. The plastisphere can protect salmonella typhimurium from UV stress under simulated environmental conditions. Environ Pollut 358, 124464. https://doi.org/10.1016/j.envpol.2024.124464.

[15] Ormsby, M.J., Woodford, L., White, H.L., Fellows, R., Oliver, D.M., Quilliam, R.S., 2024. Toxigenic Vibrio cholerae can cycle between environmental plastic waste and floodwater: implications for environmental management of cholera. J Hazard Mater 461, 132492. https://doi.org/10.1016/J.JHAZMAT.2023.132492.

[16] Ikuta, S, K., et al., 2022. Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the global burden of disease study 2019. Lancet 400 (10369), 2221–2248. https://doi.org/10.1016/S0140-6736(22)02185-7.

[17] Sharma, E., Chen, Y., Kelso, C., Sivakumar, M., Jiang, G., 2024. Navigating the environmental impacts and analytical methods of last-resort antibiotics: colistin and carbapenems. Soil Environ Health 2 (1), 100058. https://doi.org/10.1016/j.seh.2024.100058.

[18] Murray, C.J.L., et al., 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 399 (10325), 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0.

[19] Cocker, D., et al., 2023. Investigating one health risks for human colonisation with extended spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae in Malawian households: a longitudinal cohort study. Lancet Microbe 4 (7), e534–e543. https://doi.org/10.1016/S2666-5247(23)00062-9.

[20] Sartorius, B., et al., 2024. The burden of bacterial antimicrobial resistance in the WHO African region in 2019: a cross-country systematic analysis. Lancet Glob Health 12 (2), e201–e216. https://doi.org/10.1016/S2214-109X(23)00539-9.

[21] Nadimpalli, M.L., et al., 2020. Urban informal settlements as hotspots of antimicrobial resistance and the need to curb environmental transmission. Nat Microbiol 5 (6), 787–795. https://doi.org/10.1038/s41564-020-0722-0.

[22] Rigby, J., et al., 2022. Optimized methods for detecting salmonella typhi in the environment using validated field sampling, culture and confirmatory molecular approaches. J Appl Microbiol 132 (2), 1503–1517. https://doi.org/10.1111/jam.15237.

[23] Lewis, J.M., et al., 2023. Genomic analysis of extended-spectrum beta-lactamase (ESBL) producing Escherichia coli colonising adults in Blantyre, Malawi reveals previously undescribed diversity. Microb Genom 9 (6). https://doi.org/10.1099/mgen.0.001035.

[24] Asadgol, Z., Mohammadi, H., Kermani, M., Badirzadeh, A., Gholami, M., 2019. The effect of climate change on cholera disease: the road ahead using artificial neural network. PLOS ONE 14 (11), e0224813. https://doi.org/10.1371/journal.pone.0224813.

[25] Schwab, F., Gastmeier, P., Meyer, E., 2014. The warmer the weather, the more gram-negative bacteria - impact of temperature on clinical isolates in intensive care units. PloS One 9 (3), e91105. https://doi.org/10.1371/journal.pone.0091105.

[26] Malawi Meteorological Services 2024. Department of Climate Change and Meteorological Services, Malawi.

[27] Miggo, M., et al., 2023. Fight against cholera outbreak, efforts and challenges in Malawi. Health Sci Rep 6 (10), e1594. https://doi.org/10.1002/hsr2.1594.

[28] Ndungo, E., et al., 2022. Dynamics of the gut microbiome in shigella-infected children during the first two years of life. mSystems 7 (5), e0044222. https://doi.org/10.1128/msystems.00442-22.

[29] Wilson, C.N., et al., 2022. Incidence of invasive non-typhoidal salmonella in Blantyre, Malawi between January 2011-December 2019. Wellcome Open Res 7, 143. https://doi.org/10.12688/wellcomeopenres.17754.1.

[30] Heinz, E., et al., 2024. Longitudinal analysis within one hospital in sub-Saharan Africa over 20 years reveals repeated replacements of dominant clones of Klebsiella pneumoniae and stresses the importance to include temporal patterns for vaccine design considerations. Genome Med 16 (1), 67. https://doi.org/10.1186/s13073-024-01342-3.

[31] Lester, R., et al., 2022. Effect of resistance to third-generation cephalosporins on morbidity and mortality from bloodstream infections in Blantyre, Malawi: a prospective cohort study. Lancet Microbe 3 (12), e922–e930. https://doi.org/10.1016/S2666-5247(22)00282-8.

[32] Anderson, D.J., et al., 2008. Seasonal variation in Klebsiella pneumoniae bloodstream infection on 4 continents. J Infect Dis 197 (5), 752–756. https://doi.org/10.1086/527486.

[33] Kito, Y., et al., 2022. Seasonal variation in the prevalence of gram-negative bacilli in sputum and urine specimens from outpatients and inpatients. Fujita Med J 8 (2), 46–51. https://doi.org/10.20407/fmj.2021-003.

[34] Mwapasa, T. , Robertson, T. , Kazembe, D. , Mnkhwama, A. , Kalonde, P. , Feasey, N. , , and et al. 2024b. Mapping and quantifying plastic pollution in informal settlements of Urban Malawi. In prep.

[35] Adams, E.A., Byrns, S., Kumwenda, S., Quilliam, R., Mkandawire, T., Price, H., 2022. Water journeys: household water insecurity, health risks, and embodiment in slums and informal settlements. Soc Sci Med 313, 115394.

[36] Lewis, J.M., et al., 2022. Colonization dynamics of extended-spectrum beta-lactamase-producing enterobacterales in the gut of Malawian adults. Nat Microbiol 7 (10), 1593–1604. https://doi.org/10.1038/s41564-022-01216-7.

[37] Sammarro, M., et al., 2023. Risk factors, temporal dependence, and seasonality of human extended-spectrum β-lactamases-producing Escherichia coli and Klebsiella pneumoniae colonization in Malawi: a longitudinal model-based approach. Clin Infect Dis 77 (1), 1–8. https://doi.org/10.1093/cid/ciad117.

[38] Ortiz-Cortés, L.Y., Ventura-Canseco, L.M.C., Abud-Archila, M., Ruíz-Valdiviezo, V. M., Velázquez-Ríos, I.O., Alvarez-Gutiérrez, P.E., 2021. Evaluation of temperature, pH and nutrient conditions in bacterial growth and extracellular hydrolytic activities of two Alicyclobacillus spp. strains. Arch Microbiol 203 (7), 4557–4570. Available at: ⟨https://link.springer.com/article/10.1007/s00203-021-02332-4⟩.

[39] Scofield, V., Jacques, S.M.S., Guimarães, J.R.D., Farjalla, V.F., 2015. Potential changes in bacterial metabolism associated with increased water temperature and nutrient inputs in tropical humic lagoons. Front Microbiol 6, 310. https://doi.org/10.3389/fmicb.2015.00310.
[40] Guilhen, C., Forestier, C., Balestrino, D., 2017. Biofilm dispersal: multiple elaborate strategies for dissemination of bacteria with unique properties. Mol Microbiol 105 (2), 188–210. https://doi.org/10.1111/mmi.13698.
[41] Elpers, L., Deiwick, J., Hensel, M., 2022. Effect of environmental temperatures on proteome composition of Salmonella enterica serovar typhimurium. Mol Cell Proteom 21 (8), 100265. https://doi.org/10.1016/j.mcpro.2022.100265.
[42] Poimenidou, S.V., Caccia, N., Paramithiotis, S., Hébraud, M., Nychas, G.-J., Skandamis, P.N., 2023. Influence of temperature on regulation of key virulence and stress response genes in Listeria monocytogenes biofilms. Food Microbiol 111, 104190. https://doi.org/10.1016/j.fm.2022.104190.
[43] Ramamurthy, T., Ghosh, A., Pazhani, G.P., Shinoda, S., 2014. Current perspectives on viable but non-culturable (VBNC) pathogenic bacteria. Front Public Health 2 (JUL), 91118. Available at: www.frontiersin.org [Accessed: 1 August 2024].
[44] Centeleghe, I., Norville, P., Hughes, L., Maillard, J.Y., 2023. Klebsiella pneumoniae survives on surfaces as a dry biofilm. Am J Infect Control 51 (10), 1157–1162. https://doi.org/10.1016/J.AJIC.2023.02.009.
[45] Jayeola, V., Farber, J.M., Kathariou, S., 2022. Induction of the viable-but-nonculturable state in Salmonella contaminating dried fruit. Appl Environ Microbiol 88 (2). Available at: ⟨https://pubmed.ncbi.nlm.nih.gov/34731057/⟩.
[46] Trevors, J.T., 2011. Viable but non-culturable (VBNC) bacteria: gene expression in planktonic and biofilm cells. J Microbiol Methods 86 (2), 266–273 (Available at) ⟨https://linkinghub.elsevier.com/retrieve/pii/S0167701211001667⟩.
[47] Ye, C., Lin, H., Zhang, M., Chen, S. and Yu, X. 2020. Characterization and Potential Mechanisms of Highly Antibiotic Tolerant VBNC Escherichia coli Induced by Low Level Chlorination. Scientific Reports 2020 10:1 10(1), pp. 1–11. Available at: ⟨https://www.nature.com/articles/s41598–020-58106–3⟩ [Accessed: 1 August 2024].
[48] CDC. 2024. Cholera Response in Malawi.
[49] Dill-McFarland, K.A. et al. 2019. Close social relationships correlate with human gut microbiota composition. Scientific Reports 2019 9:1 9(1), pp. 1–10. Available at: https://www.nature.com/articles/s41598–018-37298–9 [Accessed: 1 August 2024].
[50] Chamas, A., et al., 2020. Degradation rates of plastics in the environment. ACS Sustain Chem Eng 8 (9), 3494–3511 (Available at) ⟨https://pubs.acs.org/doi/full/10.1021/acssuschemeng.9b06635⟩.
[51] Zhang, K., Hamidian, A.H., Tubić, A., Zhang, Y., Fang, J.K.H., Wu, C., et al., 2021. Understanding plastic degradation and microplastic formation in the environment: A review. Environ Pollut 274, 116554. https://doi.org/10.1016/j.envpol.2021.116554.
[52] Kretchy, J.P., Dzodzomenyo, M., Ayi, I., Dwomoh, D., Agyabeng, K., Konradsen, F., et al., 2020. Risk of faecal pollution among waste handlers in a resource-deprived coastal peri-urban settlement in Southern Ghana. PLoS ONE 15 (10). Available at: /pmc/articles/PMC7531843/ [Accessed: 1 December 2022.
[53] White, H.L., et al., 2023. Open defaecation by proxy: tackling the increase of disposable diapers in waste piles in informal settlements. Int J Hyg Environ Health 250. https://doi.org/10.1016/j.ijheh.2023.114171.
[54] Woodford, L., Fellows, R., White, H.L., Ormsby, M.J., Pow, C.J. and Quilliam, R.S. 2024a. Survival and Transfer Potential of Salmonella enterica Serovar Typhimurium Colonising Polyethylene Microplastics in Contaminated Agricultural Soil. Environmental Science and Pollution Research.
[55] Wierucka, K., et al., 2023. Human-wildlife interactions in urban Asia. Glob Ecol Conserv 46, e02596. https://doi.org/10.1016/j.gecco.2023.e02596.
[56] Ormsby, M.J., Woodford, L., Quilliam, R.S., 2024. Can plastic pollution drive the emergence and dissemination of novel zoonotic diseases? Environ Res 246, 118172. https://doi.org/10.1016/j.envres.2024.118172.
[57] Gerba, C.P., 2020. Microbial pathogens in municipal solid waste. In: Microbiology of Solid Waste. CRC Press,, pp. 155–174. https://doi.org/10.1201/9780138747268-5.
[58] Ormsby, M.J., Akinbobola, A., Quilliam, R.S., 2023. Plastic pollution and fungal, protozoan, and helminth pathogens – a neglected environmental and public health issue? Sci Total Environ 882. https://doi.org/10.1016/J.SCITOTENV.2023.163093.
[59] Zhu, D., Ma, J., Li, G., Rillig, M.C., Zhu, Y.-G., 2022. Soil plastispheres as hotpots of antibiotic resistance genes and potential pathogens. ISME J 16 (2), 521–532. https://doi.org/10.1038/s41396-021-01103-9.
[60] Arias-Andres, M., Klümper, U., Rojas-Jimenez, K., Grossart, H.P., 2018. Microplastic pollution increases gene exchange in aquatic ecosystems. Environ Pollut 237, 253–261. https://doi.org/10.1016/J.ENVPOL.2018.02.058.
[61] Liu, Y., Liu, W., Yang, X., Wang, J., Lin, H., Yang, Y., 2021. Microplastics are a hotspot for antibiotic resistance genes: progress and perspective. Sci Total Environ 773, 145643. https://doi.org/10.1016/j.scitotenv.2021.145643.
[62] Murray, A.K., Zhang, L., Snape, J., Gaze, W.H., 2019. Comparing the selective and co-selective effects of different antimicrobials in bacterial communities. Int J Antimicrob Agents 53 (6), 767–773. https://doi.org/10.1016/j.ijantimicag.2019.03.001.
[63] Wise, M.G., et al., 2024. Global trends in carbapenem- and difficult-to-treat-resistance among World Health Organization priority bacterial pathogens: ATLAS surveillance program 2018–2022. J Glob Antimicrob Resist 37, 168–175. https://doi.org/10.1016/j.jgar.2024.03.020.