



Do storm overflows influence AMR in the environment and is this relevant to human health? A UK perspective on a global issue

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ABSTRACT

Antimicrobial resistance (AMR) is a global public health threat, and the environment has been identified as an important reservoir for resistant microorganisms and genes. Storm overflows (SOs) discharge wastewater and stormwater, and are found throughout many wastewater networks. While there are no data currently showing the impact of SOs on the environment with respect to AMR in the UK, there is a small but growing body of evidence globally highlighting the potential role of SOs on environmental AMR. This review aims to provide an overview of the current state of SOs, describe global data investigating the impact of SOs on environmental AMR, and discuss the implications of SOs regarding AMR and human health. In addition, the complexities of studying the effects of SOs are discussed and a set of priority research questions and policy interventions to tackle a potentially emerging threat to public health are presented.

1. Introduction

Antimicrobial resistance (AMR) is a global public health threat that is predicted to cause millions of deaths per year in the coming decades (O'Neill, 2014). Approximately 1.27 million deaths globally were estimated to be attributed to antibacterial resistance in 2019 (Murray et al., 2022). AMR encompasses all settings, including clinical, agricultural, and environmental, and as such, it requires a holistic "One Health" perspective to mitigate it effectively (Velazquez-Meza et al., 2022). Many anthropogenic sources of pollution discharged into aquatic environments may contribute to the persistence, transmission, and dissemination of AMR. These include runoff from agricultural and urban land, releases from aquaculture practices, and treated and untreated wastewater (such as wastewater released during SO events, or diffuse release from septic systems) (Okonkwo et al., 2023; Singer et al., 2016, 2021).

The discharge of wastewater and stormwater through storm overflows (SOs, including combined sewer overflows (CSOs)) is common in the UK's wastewater network (House of Lords Library, 2022), as well as globally (e.g., across Europe (EurEau, 2020; Lee et al., 2022; Mahaut and Andrieu, 2019; Perry et al., 2023; Quaranta et al., 2022; Stange and Tieh, 2020), North America (Ahmed et al., 2018; Dhiman et al., 2016; Donovan et al., 2008; Eramo et al., 2017; Government of Canada, 2024; Harmon et al., 2014; Salmore et al., 2006; US Environmental Protection

Agency (US EPA), 2012; Young et al., 2013), and parts of Asia (Honda et al., 2020; Jang et al., 2021; Pan et al., 2023). They were designed to release untreated wastewater directly into water bodies when the capacity of the sewage network is exceeded, for example, following exceptionally heavy rainfall, thereby preventing the backing-up of sewage into homes and businesses (Perry et al., 2023), however, SO spilling events have been documented to occur under minimal rainfall and even under dry conditions (Hammond et al., 2021). Any SO event may allow untreated sewage – containing elevated levels of pathogenic microorganisms, antimicrobial resistant bacteria (ARB), antimicrobial resistance genes (ARGs) and chemicals and compounds with AMR selective potential – to enter the environment.

Here, we discuss the current state of SOs and provide an overview of the current knowledge on the extent to which SO discharges contribute to the burden of AMR in the environment or have potentially adverse effects on human health. We use the UK as an exemplar for the technical and policy-based issues surrounding SOs. We present knowledge gaps in the form of a series of priority research questions and policy implications. Understanding the effects of SOs on AMR in aquatic environments is necessary to evaluate the risks posed to humans who interact with the environment through recreational and occupational exposures. A graphical overview of the principles explored in this review is shown in Fig. 1.

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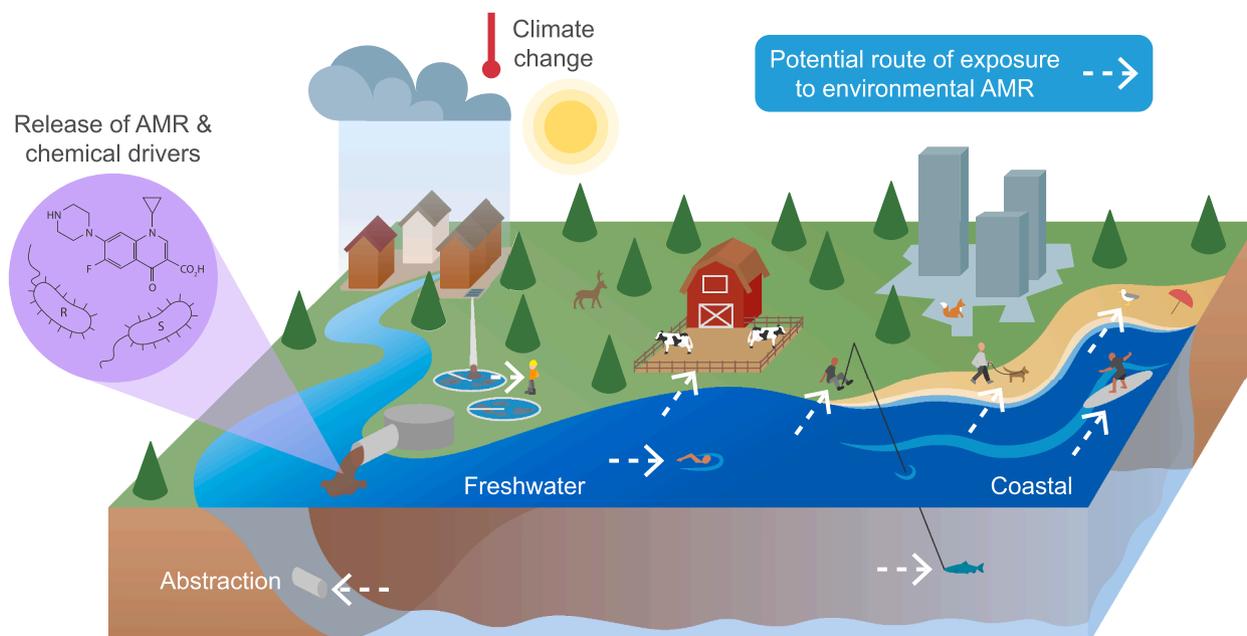


Fig. 1. Graphical overview of concepts discussed in the review. This highlights SO discharge of AMR and chemical drivers, potential exposure routes to humans of environmental AMR (indicated by white arrows), and future pressures, including increased rainfall and changes in temperature from climate change, and urbanisation.

2. Current state of SOs

2.1. UK context: a case study

Over 10 % of the UK sewage network was constructed up until (and including) 1900, and over 50 % of the network is more than 50 years old (United Utilities, 2017). Increasing populations, changing land use (e.g., greater urbanisation and increasing impermeable surfaces), and a changing climate (e.g., more frequent heavy precipitation) have meant that wastewater networks are currently under increasing pressure. It is likely that the composition of wastewater may have changed because of the increasing diversity and concentrations of pharmaceuticals, personal care products, and industrial chemicals, which could adversely impact sewage microbiology and organisms found in the receiving environment after discharge.

The water industry is regulated under the Urban Wastewater Treatment Regulations 1994 (UK Government, 1994), the Water Industry Act 1991 (UK Government, 1991), and the Environmental Permitting (England and Wales) Regulations 2016, the latter of which stipulates that regulators, such as the Environment Agency in England, must permit discharges to ensure compliance of SOs with design and water quality standards, and the protection of receiving water bodies (i.e., no deterioration in water quality from the current state) (Office for Environmental Protection (OEP), 2016). Interest in discharges from SOs has increased in recent years (e.g., from public, research (Royal Academy of Engineering, 2024), activist groups (OEP, 2021; Surfers Against Sewage, 2024; Windrush Against Sewage Pollution, 2021) and media outlets (BBC News, 2023; Cornish Times, 2023; ITV News, 2023; The Conversation, 2023; The Guardian, 2023; The Times, 2023)). Political and regulatory interest has also increased, for example, resulting in the Environment Agency calling for court fines for “serious and deliberate pollution incidents” and “prison sentences for Chief Executives and Board members whose companies are responsible for the most serious incidents” (Environment Agency, 2022b), and the UK’s Chief Medical Officer, Environment Agency and the Water Services Regulation Authority (Ofwat) releasing the opinion piece “Sewage in water: a growing public health problem” (Department of Health and Social Care et al., 2022). In August 2020, the Storm Overflows Taskforce (Department for

Environment, Food and Rural Affairs (Defra), n.d.-a), comprising representatives from the Defra, the Environment Agency, Ofwat, the Rivers Trust, the water industry, and the Consumer Council for Water, was established in England. The taskforce aimed to “develop proposals to significantly reduce the frequency and impact of sewage discharges from storm overflows with a range of ambitions from reducing spills to phasing out overflows” and “develop short term actions to accelerate progress to deliver an increased ambition on storm overflows” (Defra, n.d.-b). Furthermore, the Environment Act 2021 contains measures to alleviate the effects of SOs, which include (but are not limited to) the duty of water and sewerage companies (WaSCs) to annually publish data on SO operation, publish near-live data on discharges (within one hour of discharge beginning), and monitor water quality up and downstream of SO discharge locations (UK Government, 2021). Additionally, recent English Government policy in the Storm Overflows Discharge Reduction Plan states that “storm overflows will not be permitted to discharge above an average of 10 rainfall events per year by 2050” (Defra, 2022).

There are over 14,500 SOs within England’s sewage network (Environment Agency, 2024c) (Fig. 2), more than 3600 in Scotland (Scottish Environment Protection Agency (SEPA), 2024), over 2200 in Wales (Dŵr Cymru Welsh Water, n.d.; Hafren Dyfrdwy, 2024), and almost 2500 in Northern Ireland (Northern Ireland Water, 2024). In England, the Environment Agency requires all WaSCs to monitor discharges from SOs using event duration monitors (EDMs) and submit these data annually as part of their regulatory Annual Return (Defra, 2024). The Annual Returns cover data from overflows on sewer networks, at the inlets of wastewater treatment works (WwTWs), storm tanks at WwTWs, and pumping stations (Fig. 2). SO spill data are given as the total duration (hours) of spills and counted spills using the 12/24-hour count method. The 12/24-hour count method counts any discharge within the first 12 h of a spill as one count, and any discharges within subsequent 24-hour blocks each as one additional count, with counting not ceasing until a 24-hour block sees no discharge. For example, this would mean that a continuous or intermittent spill of 40 h duration would be equivalent to three spill counts (Environment Agency, 2018).

Data from English SOs with EDMs show a total spill duration of 3606,170 h, with a total spill count of 464,056 in 2023 (Fig. 2)

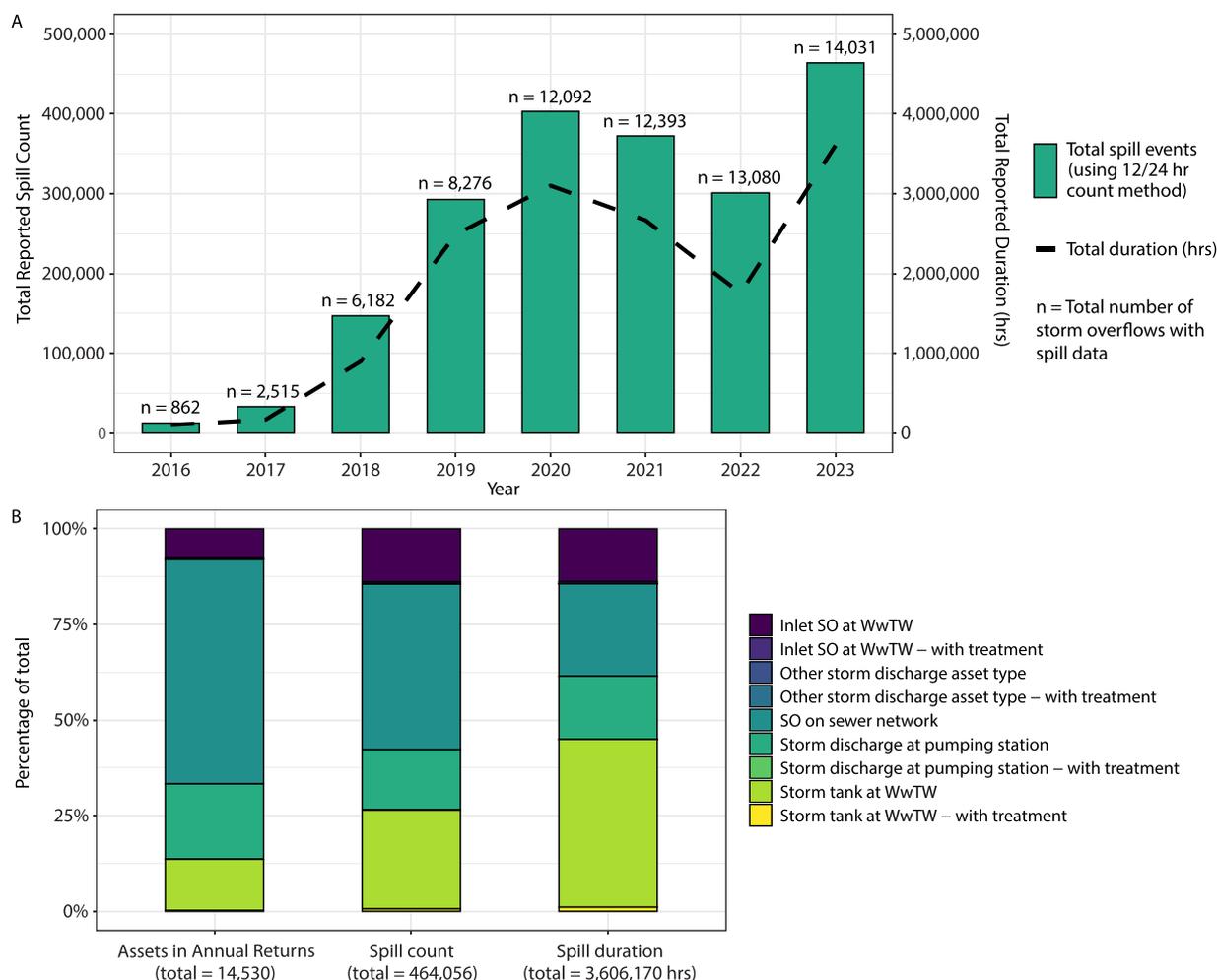


Fig. 2. Summary of Storm Overflow (SO) data for England. A) Summary of total spill count and total duration for all data in Annual Returns (all water and sewerage companies (WaSCs)) over time (data extracted from (Environment Agency, 2024a)). B) Different asset types as a percentage of the total number of assets in the 2023 Annual Returns, spill counts for 2023, and spill duration for 2023 (data extracted from (Environment Agency, 2024c)).

(Environment Agency, 2024c). Some of the highest spilling SOs in England had spill counts over 300, but for SOs that have had an EDM installed for the whole of 2023, 8 % did not spill (Environment Agency, 2024c). The total spill count for 2023 is a 54 % increase on 2022 data, which the Environment Agency and water industry have partly attributed to increased rainfall in 2023 (Environment Agency, 2024b; Water UK, 2024). Comparing spill data temporally is complicated by the fact that historically, many SOs did not have EDMs. As of December 2023, 100 % of SOs in England were fitted with EDMs (Defra et al., 2023), which is an improvement on 91 % (13,323/14,580) in 2022, 88 % (12,707/14,470) in 2021 and 83 % (12,092/14,630) in 2020 (Environment Agency, 2021; 2022a; 2023), and a vast improvement since 2016, when only 862 SOs were included in the EDM Annual Regulatory Returns (Environment Agency, 2024a). Therefore, comparing 2023 spill data, for example, to 2016 data, is problematic as the data from 2016 are likely only relating to ~6 % of SOs in England (i.e., the proportion of SOs in 2016 providing data was much lower, at 862, when it is likely that over 14,000 SOs existed based on numbers in recent years). This discrepancy in reporting data is highlighted in Fig. 2A, which indicates the total number of SOs with spill data (n).

In addition, EDMs are not always operational. Data from 2023 showed that, of the EDMs that were in operation for the whole of 2023, 31.3 % of EDMs in England were operational 100 % of the time (Environment Agency, 2024c). Further investigation showed that 80.5 % of EDMs were in operation for >90 % of the time, with only 1 %

operational for <50 % of the time, and 0.6 % not being operational for 2023 (Environment Agency, 2024c). Consequently, the actual duration and number of spills are likely to be higher than those presented in Annual Returns. Hammond et al. used machine learning to predict spill events and revealed hundreds of potentially unreported spills for over a decade at two exemplar WwTWs (Hammond et al., 2021).

Notably, spill volume is not required to be reported as part of the Annual Returns. The absence of these data makes it difficult to reflect on the possible impact of a spill on the receiving water environment, given the uncertainty over the intermittency of flow (e.g., 12/24 count method) and the intensity of the spill. The absence of volume data precludes the possibility of conducting an accurate environmental and human health risk assessment. There is also a lack of unification surrounding mitigation strategies and data availability relating to SOs across the UK and WaSCs. Current legislation only requires that EDMs be installed, and data from EDMs are provided to the Environment Agency for SOs located in England. In addition, only the English WaSCs are regulated on spill count following the Environment Act 2021 (UK Government, 2021) under the Storm Overflows Discharge Reduction Plan (Defra, 2022). Furthermore, taskforces established to tackle SO spills are country-specific (e.g., the Storm Overflows Taskforce in England (Defra, n.d.-a) and the Wales Better River Quality Taskforce in Wales (Natural Resources Wales, 2022)). To the best of our knowledge, no such taskforce exists in Scotland or Northern Ireland. WaSCs now have live or near-live spill data available in interactive maps to inform the public

Table 1
Results of a review of the global literature on AMR and SOs.

Citation	Study location	Microorganism(s) studied	Resistance type and methodology	Findings
Dewi et al. (2020)	Australia	Mixed community	Phenotypic resistance to carbapenem determined. Genotypic carbapenem resistance was determined by sequencing plasmids.	Stormwater outfall goes directly into ocean and likely had major influence on abundance of carbapenem resistant bacteria as these resistant bacteria often belonged to the same species (including species often found in sewage).
Williams et al. (2022)	Australia	Mixed community	Genotypic resistance tested by qPCR targeting <i>sul1</i> , <i>dfrA1</i> , <i>int11</i> , <i>qnrS</i> , <i>vanB</i> and <i>tetA</i> .	Resistance gene abundance of <i>sul1</i> , <i>dfrA1</i> and <i>qnrS</i> increased up to two orders of magnitude after 20.4 mm of rainfall and <i>tetA</i> increased by one order of magnitude after 40.8 mm of rainfall. Some of these genes (<i>sul1</i> , <i>tetA</i> and <i>qnrS</i>) were detected 300 m offshore after 40.8 mm of rainfall, with levels remaining high five days after rainfall event. Highest levels of ARGs in front of stormwater drains. Faecal indicator bacteria levels and sewage markers increased 10 times following rainfall.
Carney et al. (2019)	Australia	Mixed community	Genotypic resistance was tested using qPCR to target <i>int11</i> , <i>vanB</i> , <i>tetA</i> , <i>sul1</i> , <i>dfrA1</i> and <i>qnrS</i> . In addition, multiplex PCR and reverse line blot hybridisation targeting an additional 26 antibiotic resistance genes.	Absolute abundance of <i>int11</i> , <i>tetA</i> , <i>sul1</i> , <i>dfrA1</i> and <i>qnrS</i> were elevated one to two orders of magnitude after storm and modelled SO input and remained above baseline for subsequent week.
Pan et al. (2023)	China	Mixed community	Genotypic analysis of resistance gene undertaken by metagenomic sequencing.	ARGs in SO outfall were higher than in rainfall runoff. Multidrug resistance genes had the highest relative abundance. SO outfall had high pollutant and bacterial density.
Stange and Tiehm (2020)	Germany	<i>E. coli</i> and enterococci	Genotypic resistance tested by PCR for macrolide (<i>ermB</i>), trimethoprim (<i>dfrA1</i> , <i>dfrA12</i>), beta-lactam (<i>bla_{SHV}</i>), aminoglycoside (<i>aadA</i>), tetracycline (<i>tetA</i> , <i>tetB</i> , <i>tetC</i> , <i>tetK</i>), and sulfonamide (<i>sul1</i> , <i>sul2</i>) resistance.	Increase in fecal indicator bacteria associated with increase in ARGs and human-specific microbial source tracking markers 9 km away from SO after a heavy rain event.
Honda et al. (2020)	Japan	<i>E. coli</i>	Phenotypic resistance tested for ciprofloxacin, norfloxacin, tetracycline, amoxicillin, kanamycin and sulfamethoxazole/ trimethoprim.	Resistance was 3.7-log higher in SO discharges in comparison to treated effluent.
Jang et al. (2021)	South Korea	Mixed community	Resistance to tetracyclines, sulfonamides, quinolones, beta-lactams. Genes: <i>sul1</i> , <i>aac(6)-Ib-cr</i> , <i>tetX</i> , <i>blaTEM</i>	Rainfall increased ARGs by 1.9×10^3 -fold. Elevated ARGs were maintained for up to 32 h after rainfall. Increase in sewage related bacterial operational taxonomic units and ARGs suggestive of SO driving this increase.
Lee et al. (2022)	Switzerland	Mixed community	Selective plating for clarithromycin and tetracycline resistant colonies. Genotypic resistance using qPCR to test for <i>int11</i> and <i>sul1</i> and metagenomic sequencing.	SOs were the main cause of increased ARGs found in rivers during a storming event.
Eramo et al. (2017)	USA	Mixed community	Genotypic resistance using qPCR to test for sulfonamide (<i>sul1</i> , <i>sul2</i>) and tetracycline (<i>tetG</i> and <i>tetO</i>) resistance genes.	<i>Sul1</i> prevalence was found to be significantly higher in downstream surface water during wet weather when the SO was discharging, in comparison to during dry weather. However, this phenomenon was not observed for all resistance genes tested.
Ahmed et al. (2018)	USA	Faecal indicator bacteria	Genotypic resistance tested by microfluidic qPCR testing 47 target genes including ARGs, heavy metal resistance genes and genes associated with integrons were measured.	Prevalence of 27/35 ARGs greater in wet weather than in dry. Elevated faecal indicator bacteria in stormwater. Concludes that storm drain outfalls contribute microbial pollution to waters in area.
Harmon et al. (2014)	USA	Faecal coliform bacteria	Phenotypic resistance analysis to test for streptomycin, tetracycline, kanamycin, apramycin, trimethoprim and rifampicin resistance.	Most faecal coliforms could be traced to sewage or equine sources. Faecal coliform counts in samples after rainfall events were approximately one order of magnitude higher than during dry weather. Isolates were tested for phenotypic resistance.
Dhiman et al. (2016)	USA	<i>E. coli</i>	Phenotypic resistance tested for ampicillin, chloramphenicol, chlortetracycline, kanamycin, nalidixic acid, oxytetracycline, streptomycin and tetracycline.	Isolates from SO sources showed significantly greater resistance and higher multiple antibiotic resistance than from non-point sources. For isolates from the SO, 96.9 % exhibited resistance compared to 43.8 % non-point source isolates.
Salmore et al. (2006)	USA	<i>E. coli</i>	Resistance to ampicillin, chlorotetracycline, kanamycin, oxytetracycline, penicillin, streptomycin, sulfathiazole, tetracycline	Elevated <i>E. coli</i> after storms, by one to three orders of magnitude. <i>E. coli</i> levels after storms are the result of a mixture of non-human and human sources
Donovan et al. (2008)	USA	<i>Enterococcus</i> , <i>Streptococcus</i> , <i>Pseudomonas aeruginosa</i>	Phenotypic resistance testing for genatmicin, ciprofloxacin, tetracycline, nitrofurantoin, vancomycin, quinupristin-dalfopristin and erythromycin.	Pathogens isolated from river sediments. Analysed risk in three scenarios and found pathogen contaminated sediments near SO discharge could pose a health risk to individuals exposed to sediments in the mudflat areas. Resistance was tested against individual isolates but was not used in exposure risk scenario.
Young et al. (2013)	USA	Heterotrophic bacteria	Phenotypic testing for ampicillin and tetracycline resistance.	Maximum level of resistance recorded after rain event at a sampling site directly adjacent to a SO.

about spilling events. However, different WaSCs provide different data. For example, Thames Water, the first company to do so, shares the current state of all their SOs and whether they are spilling (Thames Water, 2024), as do other WaSCs (Anglian Water, 2024; Dŵr Cymru Welsh Water, 2024; Northumbrian Water, 2024; Severn Trent, 2024; United Utilities, 2024; Wessex Water, 2024; Yorkshire Water, 2024), whereas South West Water and Southern Water have maps highlighting only whether their bathing water sites are currently impacted by a sewage spill (South West Water, 2024; Southern Water, 2024).

2.2. Global context

Storm overflows are commonplace in many wastewater treatment systems globally (particularly as CSOs in cities with combined stormwater and sewer systems) (Quaranta et al., 2022). For example, estimates suggest that globally in 2020, only 57 % (by volume) of the wastewater generated by households enters sewers, and around 10 % of this is not “collected by WwTWS, most likely due to direct discharges and (in principle) combined sewer overflows” (United Nations, 2021). Sustainable Development Goal (SDG) regions with the highest proportion of wastewater generated by households entering sewer systems included Australia and New Zealand (89 %) and Northern America and Europe (86 %), with the lowest being Central Asia and Southern Asia (20 %) and Sub-Saharan Africa (17 %) (United Nations, 2021), therefore, it is likely that information relating to the state of SOs globally is largely from the former SDG regions (also reflected in the global SO data described below and global literature (Table 1)). For example, in Europe, estimates suggest that there are more than 650,000 CSOs (EurEau, 2020), whereas in the USA, CSOs are part of the wastewater infrastructure of approximately 700 communities (largely in the Northeast) (US EPA, 2024b). Large urban cities can have thousands of SOs, for example, Sydney has over 3000 “emergency relief structures” (designed overflow points) (Besley et al., 2023). Like those in the UK, overflow systems from around the world are also under pressure from the effects of climate change and heavy rainfall, population growth and ageing sewer networks (Besley et al., 2023; Roseboro et al., 2021; Wang et al., 2024), and as such, many policymakers have moved towards managing SO pollution, for example the United States Environmental Protection Agency’s Combined Sewer Overflow Control Policy under the Clean Water Act (US EPA, 2024a), or proposed revisions to the European Commission’s Urban Waste Water Treatment Directive (UWWTD) (Council Directive 91/271/EEC) (Council of the European Union, 2024; European Commission, 2022). However, some countries have no specific SO regulation, such as Ecuador, which only has restrictions on limiting the concentrations of pollutants in waterbodies, which may indirectly influence SO discharges (Montalvo-Cedillo et al., 2020).

The availability of data on both SO spills (spill count, duration, volume, real-time reporting, etc.) and discharge locations is required to fully understand the effect of SOs on AMR in the environment and human health risks. Globally, data on SO spills are varied and inconsistent, with some regions offering location-based data. For example, the US EPA CSO outfalls map gives data on locations, receiving waterbodies and compliance of CSOs, based on their National Combined Sewer Overflow Inventory (updated weekly in the Integrated Compliance Information System-National Pollutant Discharge Elimination System (US EPA, 2024b)). Other regions may offer spill count data, for example, some states in the USA (e.g., New York (New York State, 2020), New Jersey (New Jersey Department of Environmental Protection, 2022)), however, these can be inconsistently reported and/or based on modelled estimates. Other regions provide data on spill volumes, an important metric (currently inaccessible in the UK), when considering the scale of the effect from each discharge event. For example, Canada’s Open Government Portal gives monthly volume data from CSOs from 2013 to 2022 (Government of Canada, 2024). Public access to real-time spill reporting data was required in the UK under the Environment Act 2021 (UK Government, 2021), highlighting the important role that legislation

plays in data access. Globally, real-time reporting of spill data is sparse, with the UK being one of few countries providing real-time public data accessibility. Real-time spill data do exist in certain areas, for example, some cities in the USA, such as Everett in Washington, have interactive near-real-time CSO spilling maps (City of Everett, 2024).

Globally, moving towards a model where data on SO location and spills (counts, duration, volume, and real-time reporting) are publicly accessible and provided in a consistent format, will greatly improve our knowledge on the environmental and human health effects of SOs, and will inform research, policy decisions and water users.

3. Effects of SOs on AMR

Much research on the effects of different pollution sources on environmental AMR has focused on the effects of treated wastewater effluents. Wastewater contains microorganisms from a variety of sources, such as those from the human gut that reflect the health and habits of their host, which can include elevated ARGs in hosts that consume antimicrobials (Chau et al., 2022; Singer et al., 2023), or those originating from sewer network microbiomes (i.e., microbial communities associated with sewage infrastructure (Guo et al., 2019)). Wastewater also contains chemicals that can select (antimicrobials) and co-select (e.g., non-antimicrobial pharmaceuticals, personal care products, metals, and biocides) for AMR (Murray et al., 2024; Stanton et al., 2022b).

The literature on AMR in UK wastewater has grown in recent years, for example, to examine the effects of population and influent source on ARG and antibiotic load in wastewater (Elder et al., 2021), or to investigate the effects of different wastewater treatment types on AMR throughout treatment, by analysing all stages of treatment from influent to effluent and final sludges (Read et al., 2023). Research investigating the effects of wastewater effluent releases on AMR in UK rivers has found significant increases in AMR abundance in downstream sediments (Amos et al., 2018) and waters (Rowe et al., 2017). Although research has often indicated that following wastewater treatment, there is a general decrease in the abundance (absolute copies per sample volume) of many ARGs (and sometimes their prevalence (normalised copies)), this is sometimes not the case (e.g., *sulI* and *intI1* in Read et al. (2023)). Similarly, a reduction in antimicrobial load has been observed following treatment, yet the residues remaining in the effluent can reach concentrations high enough to theoretically result in selection for resistance (Hayes et al., 2022; Read et al., 2023; Singer et al., 2019). Understandably, high loads of AMR and antimicrobials have been recorded in raw, untreated wastewater (i.e., influent) (Archer et al., 2017; Elder et al., 2021; Read et al., 2023), which may be a significant component of SO discharges.

Considerable uncertainty exists regarding the dilution of microorganisms and chemicals during SO discharge. SO discharges will have varying degrees of dilution, which are dictated by variables such as the pipe diameter, speed of flow, the extent to which the dry weather flow of sewage has already filled the sewage network, and the extent of rainfall and system design (Giakoumis and Voulvoulis, 2023). In addition, the microbial and chemical composition of SO discharge will likely vary in relation to the source of the water and the wastewater catchment, and thus different SOs may have varied impacts (Giakoumis and Voulvoulis, 2023). Data on AMR in SO discharges and the effects of discharges on AMR in receiving environments are limited compared to data on wastewater influent, treated effluent, and the effects of effluent on the downstream environment. There is a distinct lack of data on AMR in/from UK SOs and a clear knowledge and data gap given the public, political, and academic interest. Similarly, data are also limited in other countries (see above and Table 1). Furthermore, current monitoring efforts of environmental AMR by regulators exist either as pilot schemes (e.g., the Environment Agency and their pilot monitoring of AMR in a select few river catchments in England as part of their PATH-SAFE programme (Schmidt, 2022)) or as an addition to existing monitoring programmes (e.g., Scottish Environment Protection Agency (SEPA) have

added AMR to their existing bathing water monitoring and have investigated the presence of cefotaxime-resistant *Escherichia coli* and vancomycin-resistant *Enterococci* (SEPA, 2023)). Table 1 presents the results of a review of the global literature on AMR and SOs. In summary, much of this research was undertaken in the USA (7 of 15 publications) (Ahmed et al., 2018; Dhiman et al., 2016; Donovan et al., 2008; Eramo et al., 2017; Harmon et al., 2014; Salmore et al., 2006; Young et al., 2013), with the remaining studies occurring in Australia, China, Germany, Japan, South Korea and Switzerland (Carney et al., 2019; Dewi et al., 2020; Honda et al., 2020; Jang et al., 2021; Lee et al., 2022; Pan et al., 2023; Stange and Tiehm, 2020; Williams et al., 2022). The majority of studies investigated resistance in either mixed microbial communities (Carney et al., 2019; Dewi et al., 2020; Eramo et al., 2017; Jang et al., 2021; Lee et al., 2022; Pan et al., 2023; Williams et al., 2022) or multiple different species (Ahmed et al., 2018; Donovan et al., 2008; Harmon et al., 2014; Stange and Tiehm, 2020; Young et al., 2013), with the remainder investigating the focal species, *E. coli* (Dhiman et al., 2016; Honda et al., 2020; Salmore et al., 2006). In general, studies found increased resistance after a storming event or rainfall, and increased resistance from SOs in comparison to treated effluent or non-point sources. A number of studies also investigated the impacts of SOs on specific groups of bacteria such as faecal indicator bacteria or pathogens, with one study investigating risk to human health from pathogens found in sediment near to a SO outlet (Donovan et al., 2008).

4. Relevance of SOs to human health

Humans are exposed to AMR in the natural environment through various exposure scenarios (e.g., bathing or other recreational activities in designated and non-designated bathing water bodies, working in agriculture or aquaculture/fisheries, and working in WwTWs (see Fig. 1)) (Stanton et al., 2022a). In addition, there is the potential for humans to be directly or indirectly exposed to AMR in wastewater-impacted environments. Stanton et al. (2022a) collated all evidence globally showing the transmission of AMR from the environment to humans, and found evidence of AMR transmission to humans from coastal bathing activities (Leonard et al., 2018), from the use of reclaimed irrigation water (i.e., treated wastewater) (Goldstein et al., 2017), and following a near-drowning incident in a river (Laurens et al., 2018). In contrast, more recent data published in 2023 found that water users were less likely to be colonised by extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales than non-water users (Farrell et al., 2023). In addition, a study investigating the impact of proximity to WwTWs in three countries found that in one of the three countries of study, those who worked at WwTWs and those who lived less than 300 m away from WwTWs were more likely to be colonised with ESBL-producing *E. coli* than the general population (Rodríguez-Molina et al., 2021). These results highlight the human health risks from exposure to treated and untreated wastewater, either from residing nearby or working in a WwTW. However, this was not observed in the other two countries from the same publication (Rodríguez-Molina et al., 2021), nor was it observed in a subsequent publication from the same study which compared ARGs in stool samples from the same cohort (Berglund et al., 2023). The variability between studies reinforces the need for more research to better understand the effects of the duration of environmental AMR exposure, exposure activities, and exposure type (e.g., treated or untreated sewage) on the risks to human health from environmental AMR.

While there are no data relating to AMR transmission to humans from exposure to SOs, other human health outcomes have been linked to SOs, particularly gastrointestinal infections. For example, Schiff et al. (2016) recorded an increase in self-reporting of gastrointestinal symptoms from surfers following ocean exposure after wet weather events, whereas Miller et al. (2022) and Brokamp et al. (2017) reported increases in visits to the emergency department for gastrointestinal symptoms following SO events. In addition, studies have modelled the

risk of contracting infections from SO-contaminated environments. For example, Donovan et al. (2008) investigated three risk scenarios to human health from exposure to SO-contaminated river sediments in the USA. Although this study tested individual isolates for resistant bacteria, these were not linked to risk scenarios.

The lack of global data surrounding SO and AMR health risk is a clear knowledge and data gap that urgently needs to be filled to allow for improved risk assessments and informed policy interventions and mitigation measures.

5. Complexities in understanding and tackling the effects of SOs

Policy and WaSC measures to alleviate the effects of SOs on the receiving environment are likely to require solutions with co-benefits (Perry et al., 2023) that are supported by a robust evidence base. Key evidence gaps need to address 1) understanding the relative contribution of varying pollution sources to the environmental burden of AMR, 2) comparing the effects of SO discharge on AMR with those of treated wastewater effluent, 3) elucidating whether there is a risk to human and animal health from AMR released from SO discharges, and 4) understanding the effects of future challenges, such as climate change, on the frequency of SO discharge and human health effects. Here, we discuss the complexities of undertaking research on these key topics.

5.1. Understanding relative contributions from differing pollution sources

The source apportionment of diffuse and point pollution sources of AMR is difficult. Catchment-based approaches can be used to investigate the effects of both single and multiple polluting inputs on receiving water bodies and assign the weight and directionality of pollution sources. Differentiating the impact in rivers from more distinct pollution sources, such as any type of human wastewater in comparison to agricultural sources, may be possible using distinct microbial genetic markers, which can be identified using microbial source tracking (Damashek et al., 2022). This can occur by targeting animal and human-specific genetic markers, such as crAssphage (Stachler et al., 2017) or *Bacteroidales* HF183 marker (Seurinck et al., 2005) for human faecal pollution, *Helicobacter* spp. Associated GFD for avian faecal pollution (Ahmed et al., 2016), and host-specific markers of the *Bacteroidales* species (e.g., HoF597 (horse), CF128 (ruminant) and PF163 (pig)) (Balleste et al., 2020). However, disentangling the effects of different sources of human wastewater – such as point sources (e.g., untreated/treated release from WwTWs) or diffuse sources (e.g., releases from septic systems) – may prove difficult using this method (see Section 5.2).

An approach often used to understand the relative contribution of different pollution sources to a waterbody is using Reasons for Not Achieving Good Status (RNAGS). Under the Water Framework Directive (WFD) (2000/60/EC) (The European Parliament and The Council of the European Union, 2000), surface water bodies are classified from “bad” to “high” status, using a series of ecological and chemical metrics. When waterbodies fail to reach the second highest status of “good”, they are assigned RNAGS. The Environment Agency data for English Water Framework Directive 2019 Cycle 3 indicate that of all RNAGS assigned to water bodies, 26 % were caused by the water industry, the second largest proportion following agriculture and rural land management practices (40 %) (Environment Agency, n.d.). RNAGS are derived from the average condition of a water body, which may result in minimising or missing the acute effects of localised SO spilling incidents. Furthermore, although using Water Framework Directive status is useful for assessing the ecological and chemical status of a water body, it does not include metrics to assess the potential risks related to AMR, pathogens, or use molecular approaches, such as microbial source tracking, as described above. RNAGS were intended to focus the regulator on the greatest sources of pollution within a catchment, but in practice, they can be found to be used by different sectors to deflect blame, which is

likely to hinder collaboration and progress between polluting actors.

A lack of understanding surrounding the relative source of pollution inputs within river catchments hampers mitigation efforts. Aquatic environments contain a complex mixture of microorganisms and antimicrobial resistance driving chemicals representing different sources. A significant challenge is disentangling these under current and future climate and population scenarios.

5.2. Comparing the different effects of SO discharge and treated wastewater effluent on AMR

Disentangling the effects of SOs from other similar polluting inputs (such as treated wastewater), was recently highlighted as a critical knowledge gap in a recent United Nations Environment Programme report (UNEP, 2023). However, doing so may prove difficult as discharge locations can be co-located and the microbial profiles of each may be similar. Further, pollution levels from SOs are inconsistent during a spilling event, therefore providing more complexity to the issue. The “first flush” phenomenon describes the initial phase of a SO event where high loads of various pollutants may be released (Gupta and Saul, 1996), including those from pipe biofilms and sediments that might have accumulated over time in the sewer network (Li et al., 2019). The first flush phenomenon has been investigated for numerous chemical pollutants, such as nitrogen, phosphorus and various heavy metals such as lead and zinc (Barco et al., 2008; Peng et al., 2016). A 2015 study found that *E. coli* loads in SO releases were 24.5 times higher in the rising limb compared to the falling limb for a given flow rate, and that generally, loadings of *E. coli*, total suspended solids and wastewater micropollutants increased rapidly with flow rate (Anne-Sophie et al., 2015). Therefore, it is conceivable that the first flush of sewer pipe biofilms and sediments may result in an elevated release of ARB and ARGs, adding another layer of complexity to understanding the role that SO releases play in driving AMR downstream, particularly in the absence of flow and volume spill data.

Co-location of SO and treated discharge outlets and their similar microbial profiles can constrain differentiating these sources using methods such as microbial source tracking of human waste-related genes (see above). However, some publications have identified different microorganisms or micropollutants indicative of SO pollution. From dry and wet weather sampling of the Kanda River in Tokyo, Ekhlas et al. (2021) found that *Bacteroides* spp. and *Arcobacter* spp. significantly increased in abundance during a CSO event, and suggested *Bacteroides* spp. may be used to indicate human faecal pollution, whereas *Arcobacter* spp. may be associated with sewer pipes. Phillips et al. (2012) quantified the relative contribution of a WwTW in the USA to the micropollutant load in the receiving lake, and found that the concentrations of some hormones and many micropollutants in CSO releases were up to 10 times higher than those from treated effluent. Further, chemical markers have been suggested as useful indicators of the presence of raw sewage, including caffeine (Buerge et al., 2006; Munro et al., 2019), caffeine/sucralose ratios (Cantwell et al., 2018), cocaine, bezafibrate, sulfapyridine, benzoylcegonine, diazepam and furosemide (Munro et al., 2019).

There remains a need to elucidate the relative effects of SO and treated effluent discharge on AMR in the environment. This necessitates identifying key methodologies to apportion these sources, which will provide the data needed to inform mitigative action.

5.3. Relative risk of SOs to human health

Elucidating the effects of SOs on human health concerning AMR comes with many complexities, including difficulties in establishing causal linkages between resistance found in the environment and a resistant infection, understanding source apportionment with regard to colonisation, and various challenges surrounding study design.

Establishing a causal link between exposure to a pathogenic microorganism in the environment and negative clinical outcomes is difficult

to demonstrate, and typically relies on studying user groups that have naturally higher exposure to polluted water (e.g., surfers (Leonard et al., 2015)). This is further complicated when considering resistance, given the mobility of ARGs. When humans are exposed to pathogens, their symptoms often appear relatively quickly after exposure meaning that causal linkages based on their recent history (e.g., travel to a foreign country (Chen and Blair, 2015), eating a certain type of food (Tuffs, 2011) or exposure to faecally contaminated waters (Chen and Blair, 2015; Wade et al., 2022)) can be more readily established. Understanding this for AMR is more complex, as humans may be exposed to and become colonised by AMR commensal, opportunistic, or environmental organisms, which may never result in a clinical outcome. However, because bacteria can pass their genes to distantly related species via horizontal gene transfer (Barlow, 2009), transfer of resistance from commensal organisms to pathogens may occur, resulting in clinical failure (Stanton et al., 2022a). The mobility of resistance can make it nearly impossible to establish a causal link with environmental exposure. Furthermore, given that resistance could have come from a large range of different microbial hosts, and therefore sources, it can be difficult to link human acquisition of resistance to a particular pollution source. As with studies investigating gastrointestinal illnesses, one solution to these research limitations could be to study the longitudinal effects of AMR in the gut microbiomes of potentially exposed groups, such as wild swimmers, downstream of SOs. However, because SOs and effluent pipes can be co-located, it may be difficult to disentangle their respective impacts.

The current evidence base for attributing increased carriage of ARGs in humans following exposure to SO releases is lacking, largely as a result of 1) the difficulty in conducting such a study at the appropriate scale to gain confidence in the causal association, and 2) the co-association of SOs with other sources of pollution, including treated wastewater, and urban and agricultural runoff. There remains a need to understand the thresholds of pathogens and ARGs in aquatic environments, above which there is a measurable increased risk to human acquisition and carriage following different exposure routes. In the interim, efforts must be made to minimise pathogen and ARG contamination of the aquatic environment, not only through SOs but also through treated sewage and diffuse sources where applicable.

5.4. Future challenges

The impact of SOs on AMR and their risk to environmental and human health is likely to change in the coming years as a result of many factors. These may include a changing climate (e.g., increased temperatures and frequency of heavy precipitation), increases in population, and changes in land use.

According to the Met Office, in the UK, climate change is predicted to cause warmer and wetter winters, hotter and drier summers as well as more frequent and intense weather events, including intense rainfall in summer months (Met Office, n.d.). Increased rainfall, in both winter and summer, from intense weather events may cause an increase in discharges of untreated sewage into downstream water environments, as sewerage networks may reach capacity more frequently. In addition, the impact of warmer conditions resulting from climate change may result in an increased prevalence of AMR infections. For example, in the USA, MacFadden et al. (2018) found higher rates of three resistant clinical pathogens in states with warmer minimum temperatures when investigating the association between AMR and local temperature across different states. This, therefore, may result in more ARB and ARGs entering the sewerage network and potentially being discharged into downstream water environments during SO events. Derx et al. (2023) modelled the risk of infection from enterococci, *Giardia*, and *Cryptosporidium* from the recreational use of a river downstream of SOs under different climate change models. The study determined an increased risk of contracting these infections from SOs in future climate scenarios, suggesting that sustainable water management is required to prevent

transmission events.

Increasing populations will require an increase in housing, which may not be met with an increased sewage network capacity. In addition, greater urbanisation may lead to an increase in impermeable land if Sustainable Drainage Systems are not implemented (Perry et al., 2023). Therefore, engagement with stakeholders involved in approving new housing is necessary to ensure potential sewerage capacity issues are avoided and that sustainable drainage solutions are sought.

6. Priority research questions

Several knowledge gaps hamper risk assessment, policy making, and informed mitigation efforts of the risks posed by the release of AMR and AMR-driving chemicals from SOs into the environment, and the potential downstream risks to human health. These knowledge gaps are presented below as priority questions for research.

6.1. Surveillance of AMR in/from SOs

At the time of writing, there has been no research on AMR from UK SOs. A better understanding of the composition, concentrations, total loads, and types of pollution (including AMR and AMR-driving chemicals) released from SOs – and the effects of first flush and biotic factors (e.g., dilution effects) on these – is necessary to elucidate the ecological and health impacts.

6.2. Pollution source apportionment

The sources of pathogens and ARGs include point sources (e.g., wastewater and industrial discharge) and diffuse sources (e.g., urban and agricultural runoff). However, when these sources are mixed into a river at the catchment scale, it becomes very challenging to apportion the signal. It is critical to understand the relative risk that different pollution sources pose to increasing AMR levels in downstream environments. Understanding the contribution of the burden of AMR from SOs and other pollution sources will allow for the implementation of data-driven mitigation strategies by prioritising pollution sources.

6.3. Potential risk to human health

Understanding the risk posed by environmental AMR to human health is an area of growing research interest. Although data exists, it is currently extremely limited. Certain leisure activities in the UK (e.g., wild swimming (Bates and Moles, 2022)) are increasing in popularity, potentially exposing individuals to AMR in the environment. The degree to which a particular person is at risk and how that risk varies with age, health, exposure dose, quantity of pathogen and/or AMR present, and environmental conditions, is largely unknown and potentially highly variable. Improving this understanding is essential for risk assessment and mitigation efforts, including for engineering and social solutions.

7. Priority policy interventions

From this review, we identified a list of priority focus areas for policy interventions that will allow further understanding of the role SOs play in disseminating AMR and the potential risk posed to human health.

7.1. Improvement in SO reporting

The provision of EDM and real-time spilling data has been a positive step towards beginning to understand the scale of the effects of SO discharges on the environment and human health in the UK. However, the statutory reporting of SO spills via the Annual Returns process requires improvements to allow researchers, policymakers, the public, and WaSCs to draw accurate conclusions from the data. These improvements include ensuring that EDMs work 100 % of the time and, most

importantly, including spill volume as a required metric in the Annual Returns.

7.2. Regulatory surveillance of AMR in wastewater and the environment

Currently, there is no legislation requiring the surveillance of AMR in natural environments or wastewater settings in the UK. Current monitoring efforts of environmental AMR by regulators exist either as pilot schemes (e.g., the PATH-SAFE programme (Schmidt, 2022)) or additions to existing schemes (e.g., SEPA's bathing water monitoring (SEPA, 2023)). Whilst scaling up an AMR monitoring scheme from scratch is an expensive task that will take time, amending existing schemes, such as incorporating it into bathing water monitoring or using frameworks and networks for wastewater monitoring, could help facilitate the establishment of such a scheme.

8. Conclusions

As evidenced throughout this review, based on the global literature, it is likely that SO discharges contribute to elevated levels of ARB and ARGs in downstream aquatic environments. This could pose an increased risk of AMR acquisition in humans who interact with such environments through recreational or occupational exposures. However, with the lack of data on human exposure to SO-polluted environments regarding AMR, it is unclear to what extent this is happening in the UK and globally. Research into the effects of SOs on AMR in the environment and potential associated public health risks, along with policy action to improve SO reporting data (e.g., inclusion of volume spilt) and undertake surveillance, would enable a greater understanding of potential risks and where mitigation may be best implemented.

CRedit authorship contribution statement

Holly J. Tipper: Conceptualization, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Isobel C. Stanton:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **Rachel A. Payne:** Data curation, Writing – review & editing. **Daniel S. Read:** Conceptualization, Funding acquisition, Writing – review & editing. **Andrew C. Singer:** Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Daniel S. Read reports financial support was provided by Natural Environment Research Council. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Any data gathered from other publications is referenced in text and bibliography.

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