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Isolation and biocontrol of bacteriophages from wastewater in the city of Lomé, Togo: potential application as a novel source for antimicrobial therapy

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Abstract:

Background: Bacteriophages offer one of the most promising solutions to the challenges of antimicrobial resistance in bacteria. The aim of this study is to investigate bacteriophages as a source of new antimicrobial therapy.
Methodology: Waste water samples were randomly collected from 8 different locations in the city of Lomé for bacteriophage isolation. The phages were isolated using multi-resistant clinical isolates (*Escherichia coli* 1642 and *Staphylococcus aureus* 0868) as hosts by means of a spot test. The host range of the phages was determined also by a spot test using 8 other clinical bacterial isolates including two reference strains (*E. coli* ATCC 25922 and *S. aureus* ATCC 29213). The virulence of the phages and their effects on bacterial growth were assessed by *in vitro* experiments using *E. coli* 1642 BBec phage suspension.
Results: Isolation of phages by the spot test was positive only with the host *E. coli* 1642. A reduced host range was observed with the other bacteria. The BBec phage suspension showed a titer of 1.6×10^7 PFU/ml. Virulence studies revealed a latency time of less than 10 minutes, a degree of absorption of 87% and a burst size of 63 PFU/cell. The effect of BBec phage suspension on *E. coli* 1642 showed an almost total reduction in the population of *E. coli* 1642 after 4 hours.
Conclusion: This study provided scientific data showing the antibacterial effect of a phage suspension (BBec) on a multi-resistant clinical isolate of *E. coli* 1642. This phage could therefore be explored as a candidate for the development of new antibacterial therapies.

Keywords: multidrug resistance, bacteriophage, antibacterial effect, wastewater, Togo

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Isolement et biocontrôle des bactériophages à partir des eaux usées de la ville de Lomé, Togo: potentielle application en tant que source de nouvelles thérapies antimicrobiennes

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Résumé:

Contexte: Face aux problèmes de multirésistance des bactéries aux agents antimicrobiens, les bactériophages représentent l'une des solutions les plus prometteuses. L'objectif de ce travail est d'étudier les bactériophages en

tant que source de nouvelles thérapies antimicrobiennes.
Méthodologie: Des échantillons d'eaux usées ont été collectés de manière aléatoire dans 8 endroits différents de la ville de Lomé pour l'isolement des bactériophages. Les phages ont été isolés en utilisant comme hôtes, des isolats cliniques (*Escherichia coli* 1642 et *Staphylococcus aureus* 0868) multirésistants par le biais d'un test ponctuel. La gamme d'hôte des phages a également été déterminée par un test ponctuel utilisant 8 autres isolats dont deux souches de référence (*E. coli* ATCC 25922 et *S. aureus* 29213). L'évaluation de la virulence des phages et leurs effets sur la croissance des bactéries ont été réalisés à travers des expérimentations *in vitro avec* une suspension de phages d'*E. coli* désignée BBec.
Résultats: L'isolement des phages par le test ponctuel s'est révélé positif seulement avec l'hôte *E. coli* 1642. Une gamme d'hôte réduite a été observée avec les autres bactéries. La suspension de phage BBec a présenté un titre de 1,6 x 10^7 UFP/ml. L'étude de sa virulence a révélé un temps de latence inférieur à 10 minutes, un degré d'absorption de 87% et une taille de rafale de 63 UFP/Cellule. L'effet de la suspension phagique BBec sur l'isolat *E. coli* 1642 a montré une réduction quasi totale de la population de l'isolat *E. coli* 1642 au bout de 4 h.
Conclusion: Cette étude a permis de fournir des données scientifiques qui montrent l'effet antibactérien d'une suspension de phage (BBec) sur un isolat clinique multirésistant *E. coli* 1642. Ce phage pourrait donc être exploré comme candidat au développement de nouvelles thérapies antibactériennes.

Mots clés: multirésistance aux médicaments, bactériophages, effet antibactérien, eaux usées, Togo.

Introduction:

Antibiotics have been important therapeutic discovery for human health. Their use has long helped to reduce mortality and morbidity worldwide. However, the increased use and misuse of these antimicrobial agents have resulted in the emergence of resistance in microbial populations counteracting the beneficial effects of antibiotics (1). Thanks to their genetic flexibility and plasticity, pathogenic bacteria are capable of setting up specific resistance mechanisms against a particular antibiotic. In fact, some strains are able to establish multiple resistance to several antibiotics at the same time, giving rise to what is known as multidrug resistant (MDR) bacteria (2).

According to the World Health Organization (WHO), MDR bacteria are one of the most serious threats to global health, food security and development. It is now reaching dangerously high levels in all regions of the world. New resistance mechanisms are emerging and spreading worldwide, compromising our ability to treat common infectious diseases. The MDR bacteria cause serious infections that are difficult or impossible to treat, due to the loss of effectiveness of antibiotics.

The UK Government's Review on Antimicrobial Resistance published in 2014 estimated that up to 10 million people could die each year from AMR infections by 2050 (3). However, in 2019 antibiotic-resistant bacteria directly caused the death of more than 1.2 million patients (4). The situation is alarming in countries with limited resources where infectious diseases, poverty and malnutrition are endemic (5). In sub-Saharan West Africa, 27.3 deaths per 100,000 population (all ages) were attributable to antibiotic-resistant germs (4). The six main pathogens responsible for these resistance-associated deaths are *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii* and *Pseudomonas aeruginosa* (4,6).

Faced with this growing problem, the search for a new antimicrobial strategy has become one of the highest priorities of modern medicine and biotechnology. Bacteriophages (environmental viruses that naturally infect bacteria) represent one of the most promising solutions (7). In fact, the idea of using bacteriophages to treat bacterial infections (phage therapy) is not new. At the beginning of the 20th century, phage therapy enjoyed successes that were to be the starting point of a true "globalisation" (8). However, with the discovery of penicillin in 1928 and the second World War, which generated an immense need for anti-infective treatments, phagotherapy was gradually abandoned in favour of antibiotic therapy (9). Today, with the emergence of bacteria that are multi-resistant to antibiotics, there is renewed interest in the study of bacteriophages (10).

Recent studies showed that bacteriophages are currently used in the food industry to limit the development of pathogens during processing and/or on surfaces in contact with food. In agriculture, they are used to combat plant pathogenic bacteria (11) and also in human medicine, to treat patients suffering from infection by multi-resistant bacteria (12). This study focuses mainly on the isolation of bacteriophages from the environment and the potential application in the biocontrol of MDR bacteria.

Materials and method:

Study setting:

This study was carried out in Lomé, Maritime region of Togo, which included Bè Lagoon (1.24113176; 6.14959641), West Lake (1.21255321; 6.13774805), Forever Reservoir (1.22217439; 6.16634648), Tokoin Séminaire (1.21201485; 6.15123999), Tokoin Dogbeavou (1.21300797; 6.15174332), Bè Beach (1.2492 3218; 6.1308177), Oando Beach (1.22930563; 6.12336644) and Palm Beach (1.22196697; 6.12 061692).

The Laboratory of Biomedical, Food and

Environmental Health Sciences, High School of Biological and Food Technics at the University of Lomé provided the setting for sample handling.

Bacteria strains:

The bacterial strains used in this study were supplied by the Medical Bacteriology Department of the National Institute of Hygiene of Lomé, Togo. A total of 10 strains were used which include 8 clinical isolates from various samples (urine and pus) and 2 reference strains (*Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213).

Sample collection:

Waste water samples were randomly collected on the 15th July 2022 from a total of 8 locations; 5 samples of urban wastewater from Bè Beach (BB), Oando Beach (OB), Palm Beach (PB), Tokoin Séminaire (TS), Tokoin Dogbeavou (TD), and 3 other samples from Bè Lagoon (BL), West Lake (WL) and Forever Water Reservoir (FR). Each sample was collected in a sterile 50 ml bottle. For each sample, the bottle was first rinsed with wastewater before being filled to 30 ml. After collection, the samples were transported to the laboratory as quickly as possible in a pot of ice for bacteriophage isolation (13).

Bacteriophage isolation:

The phage enrichment method was used for the isolation of bacteriophages against the host bacterial strains (*E. coli* 1642 and *S. aureus* 0868) (14). Briefly, under aseptic conditions, 5 ml of overnight-grown bacterial cultures of each host were mixed with 5 ml of wastewater samples from each site. Each mixture was then incubated for 24 h at 37°C in a shaking incubator (70 rpm) with 10 ml of Luria-Bertani (LB) broth in sterile 50 ml bottles. Each mixture was centrifuged at 6,000g for 15 mins. The supernatant was collected in a syringe and filtered directly into 1.5 ml Eppendorf tubes using a 0.20 µm syringe filter. The tubes were stored at 4°C.

The filtrate (phage suspension) was tested for phage activity using the spot test. Briefly, a 5 ml suspension of overnight grown bacterial cultures (each host bacterium) was inoculated by flooding onto LB agar and incubated for 15 mins. Subsequently, 10 µl of each filtrate was deposited on the lawn of the corresponding host bacterium and allowed to dry. For the control, 10 µl of sterile water was also deposited. Each LB plate was then incubated for 24 hours and the appearance of clear spots (plaques) indicated the presence of bacteriophages in the filtrate. This test was performed on the other clinical bacterial isolates as well as on the reference strains of *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 to measure the host range of the phage suspensions. The diameters of the lysis plaques were measured and the data were graphically synthesised to evaluate the lytic capacity of the phage suspensions according to location.

Bacteriophage titration:

The suspension was serially diluted (10^0 to 10^{-8}) with magnesium sulphate buffer (25 ml Tris-HCl, 1 g $MgSO_4$, 2.9 g NaCl, 500 ml H_2O) (14). Prior to this, a 5 ml suspension of overnight grown bacterial cultures of each bacterium was inoculated onto the LB agar by flooding. After 15-20 min of incubation, 10 µl of each dilution was applied to the surface of the agar and allowed to dry. The plates were then incubated at 37°C for 24 h and the titre was obtained by counting the lysis spots observed with the dilution causing the most lysis on the agar using the formula (15); T(pfu)n = (N x F)/V(ml), where T is the titre, 'pfu' is phage format unit, N is the number of phages counted, F is the dilution factor, and V is the volume of phage suspension deposited.

One-step phage multiplication kinetics:

Phage cycle characterisation was performed using BBec suspension and *E. coli* 1642 host. Briefly, 100 µl of bacterial culture (1.5 x 10^8 CFU/ml, *E. coli* 1642) was infected with 100 µl of *E. coli* 1642 BBec suspension in 10 ml of LB broth contained in a sterile 50 ml bottle and incubated at 37°C for 10 mins. The mixture was centrifuged at 12,000 rpm for 5 mins and the supernatant was collected for titration and estimation of the percentage of phages absorbed on the cell surface in 10 mins (degree of absorption) according to the formula (16); DA=[pfu(0)-pfu(10)]/pfu(0) x 100, where DA is the degree of absorption and 'pfu' is the phage format unit.

The pellet containing the infected cells is resuspended in 10 ml of LB broth and incubated at 37°C. An aliquot of 100 µL is taken every 10 mins for 50 mins to titrate the phages. The data obtained, representing the evolution of the phage titre of the culture as a function of time, were synthesised in the form of a graph. The latency period was determined from the graph and the number of phages released per cell was calculated by dividing the maximum viral titre by the initial titre (t=0 min).

Evaluation of the effects of phage on bacterial growth:

A mixture of 100 µl of an 18 h suspension culture of *E. coli* 1642 was added to 10 ml of LB broth and incubated for 10 mins before the addition of 100 µl of the BBec suspension (17). This preparation was carried out in 6 sterile 50 ml bottles labelled (B+P, 0 h); (B+P, 2 h); (B+P, 4 h); (B+P, 6h.); (B+P, 24 h) and (B+P, 48 h) respectively. In parallel, positive controls were prepared under the same condi-

tions by adding 100 µl of overnight-grown bacterial cultures of *E. coli* 1642 to 10 ml of LB in a sterile 50 ml bottle; this preparation was also prepared in six sterile 50 ml bottles labelled (B, 0 h); (B, 2 h); (B+P, 4 h); (B, 6 h); (B, 24h) and (B, 48h).

The optical density (OD) of the flasks (B + P, 0 h) and (B, 0 h) was measured directly at 600 nm. The other flasks were placed in a shaking incubator. After two (2) hours of incubation, the flasks (B+P, 2 h) and (B, 2 h) were removed from the incubation and their ODs were measured at 600 nm. This procedure was repeated with the flasks ((B+P, 4 h), (B, 4 h)); ((B+P, 6 h), (B, 6 h); ((B+P, 24 h), (B, 24 h)) and ((B+P, 48 h), (B, 48 h)) respectively after 4, 6, 24 and 48 h of incubation. The experiment was repeated three times and the data obtained are summarised in the form of graphs where each point corresponds to the average of the OD_{600} nm values of the three measurements of each group of hours.

Statistical analysis of data:

Data were entered in Microsoft Excel 2019 MSO (Version 2209 Build 16.0.15629. 20152) before being exported to GraphPad Prism8 software for statistical analyses. Means were compared using the two-way ANOVA. P-value of less than 0.05 was considered statistically significant.

Results:

Isolated phages:

In all, 16 phage suspensions (8 from *E. coli* 1642 and 8 from *S. aureus*) were obtained from the water samples and recorded as shown in Table 1. The presence of bacteriophages was detected by the spot test. Lysis plaques ranging in diameter from 9 to 11 mm were observed in the spot test results of the 8 filtrates obtained from *E. coli* 1642 phage suspensions (Fig 1A). The other 8 filtrates obtained from *S. aureus* 0868 phage suspensions did not show lysed plaques (Fig 1C).

The host range results for *E. coli* 1642 and *S. aureus* 0868 phage suspensions are shown in Table 2. No interaction (lysis zone) was observed between the suspensions obtained from *E. coli* 1642 and the other bacteria, whereas for the suspensions obtained from *S. aureus* 0868, an interaction was observed only with the isolate *S. aureus* 0931 (Fig 1B). This indicates that these suspensions have a very restricted host range.

Table 1: *Escherichia coli* 1642 and *Staphylococcus aureus* 0868 phage suspensions from different samples

Hosts	**Phage suspension**							
Escherichia coli 1642	BBec	OBec	PBec	TSec	TDec	BLec	WLec	FRec
Staphylococcus aureus 0868	BBsa	OBsa	PBsa	TSsa	TDsa	BLsa	WLsa	FRsa

BB = Bè Beach, OB = Oando Beach, PB = Palm Beach, TS = Tokoin Séminaire, TD = Tokoin Dogbeavou, BL = Bè Lagoon, WL = West Lake, FR = Forever water reservoir, ec = *E. coli* 1642 phage suspensions, sa = *S. aureus* 0868 phage suspensions

Table 2: Bacterial host range of bacteriophage suspensions from wastewater samples in the city of Lome, Togo

Bacteria strains	**Ec**	**Sa**
Escherichia coli 1628	N	N
Escherichia coli 1610	N	N
Escherichia coli 1555	N	N
Escherichia coli ATCC 25922	N	N
Staphylococcus aureus 0931	N	P
Staphylococcus aureus 4031	N	N
Staphylococcus aureus RM	N	N
Staphylococcus aureus ATCC 29213	N	N

Ec = *E. coli* 1642 phage suspensions, Sa = *S. aureus* 0868 phage suspensions, N = indicates that there is no phage-bacteria interaction, P = indicates that there is a phage-bacteria interaction.

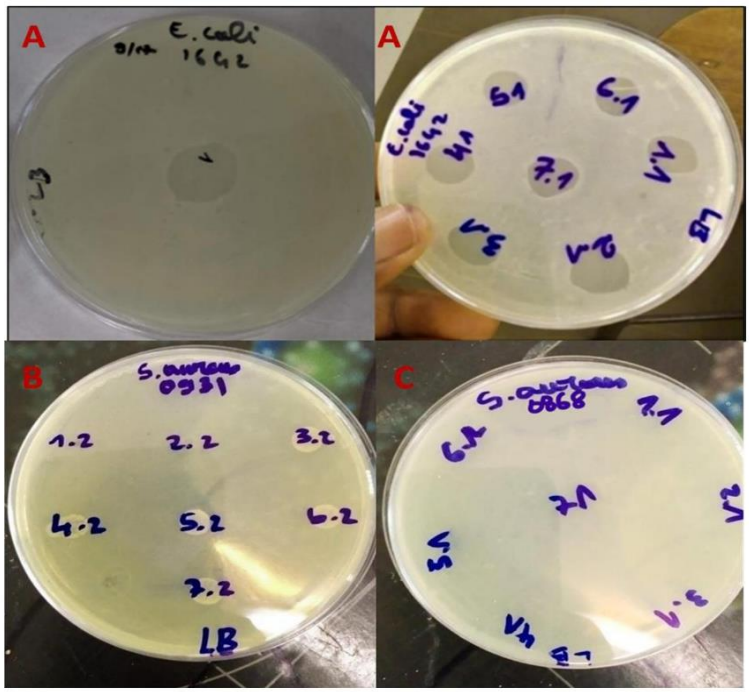

Fig 1: Spot test

A: Spot test using *E. coli* 1642, B: Spot test using *S. aureus* 0931, C: Spot test using *S. aureus* 0868

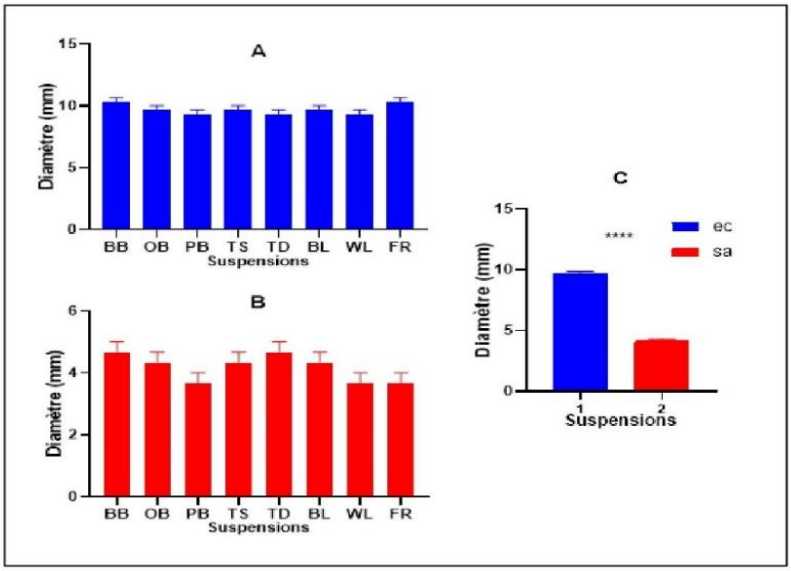

Figure 2: Lytic capacity of phage suspension

BB = Bè Beach, OB = Oando Beach, PB = Palm Beach, TS = Tokoin Séminaire, TD = Tokoin Dogbeavou, BL = Bè Lagoon, WL = West Lake, FR = Forever water reservoir, ec = *E. coli* 1642 phage suspensions, sa = *S. aureus* 0931 phage suspensions

Fig 2 shows in A the diameters of lysis plaques caused by *E. coli* 1642 phage suspensions (ec) from the different samples, in B the diameters of lysis plaques caused by *S. aureus* 0931 phage suspensions (sa), and in C, the set of lysis plaque diameters caused by the two phage suspensions. The differences in the mean diameter of the lysis plaques caused by the suspensions in A are not statistically significant (p=0.42). However, in C, mean differences between "ec" and "sa" are statistically significant (p<0.0001).

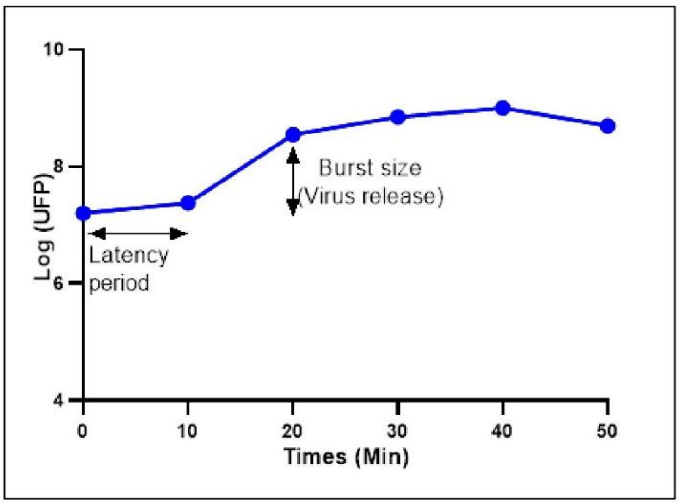

Fig 3: Kinetics of the multiplication of the *E. coli* 1642 BBec phage suspension

Table 3: Virulence data of *Escherichia coli* 1642 BBec phage suspension

Settings	Results
Degree of absorption (%)	87
Burst size (virus release) (UFP/Cell)	63
Latency (mn)	< 10

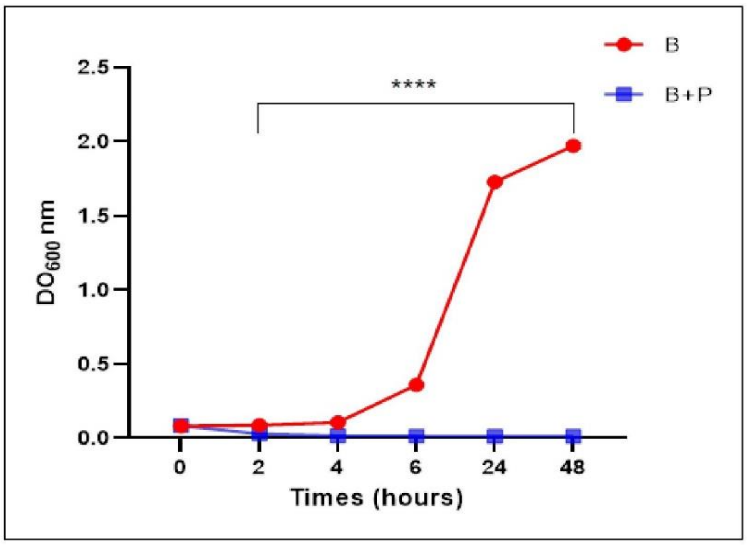

Fig 4: Evolution of the *E. coli* 1642 population

B+P: suspension of BBec mixed with a suspension of *E. coli* 1642 in LB broth, B: Suspension of *E. coli* 1642 alone in LB broth (control positive)

The titer of the BBec suspension obtained after counting and calculation is 1.6×10^7 PFU/ml. The kinetics of the one-step multiplication of the BBec phage are shown in Fig 3 and the virulence parameters are shown in Table 3. The effect of BBec suspension on the growth of *E. coli* 1642 is shown in Fig 4. The evolution of the optical density is used to indicate the population of *E. coli* 1642. The optical density (OD) of the control, absence of BBec suspension (B), increases until t = 48 hours, while that of the test, presence of BBec

suspension (B+P), gradually decreases until t = 4 hours, when it is close to zero. Comparing (B) and (B+P), there is a significant increase in the OD of (B) compared to OD (B+P) over time. From t = 2 hours to t = 48 hours, a statistically significant difference (p<0.0001) was observed between the OD values of the (B) and (B+P) groups.

Discussion:

Several studies have reported isolating bacteriophages from wastewater (18,19). They are usually detected by lytic activity on the host bacteria. In this study, phage isolation from clinical isolates of *E. coli* 1642 and *S. aureus* 0931 with reduced lytic spectrum from wastewater was demonstrated by the spot test and host range. Phage suspensions "ee" and "sa" obtained from different sites all exhibited lytic activity against *E. coli* 1642 and *S. aureus* 0931 respectively. These sampling sites would be contaminated by these pathogens (*E. coli* 1642 and *S. aureus* 0931) because according to Son et al., (20), phages are known to be present wherever hosts are present. This observation may be due either to resistance of these bacteria to bacteriophages, or to the absence of lytic phages specific for these bacteria in the suspension, or even to an insufficient incubation time to allow adsorption of the phages on the cell membrane of these bacteria.

On the basis of the "kill the winner" theory, we may assume that *E. coli* 1642 and *S. aureus* 0931 were isolated from the samples with a low density of lytic phages specific for these bacteria or with a low presence or absence of these bacteria. This theory suggests that phages adapt to preferentially infect the bacterial line with the highest frequency in the population (21). Unfortunately, there are no previously documented reports on the prevalence of these bacteria in these ecosystems. The work of Mirzaei and Nilsson (22) showed that a cocktail of 6 phages isolated from waste water was able to lyse 55 strains of *E. coli*. In addition, obtaining bacteriophages with a broader or different spectrum than that of their host bacterium is challenging because if a bacteriophage targeting proteins are modified, it may lose the ability to recognise its host. As a result, there will be no replication or maintenance of the bacteriophage in its host (23). Evaluating the lytic capacity of phages by measuring the diameter of the lysis plaque showed an independence between lytic capacity and sampling sites. On the other hand, host dependence is very real.

The titer of 1.6 x 10^7 PFU/ml of BBec suspension obtained by counting lysis plaques is within the average range of phages found in liquid media, between 10^7 and 10^8 PFU/ml (8). *S. aureus* phages isolated from urban wastewater and the Ebrié lagoon in Côte d'Ivoire showed titers between 10^5 and 10^8 PFU/ml in the study of Addablah et al., (19). The demonstration of BBec phage replication in the presence of *E. coli* 1642 provided virulence data such as a latency period of less than 10 mins, a degree of uptake of 87% in 10 minutes and an estimated burst size of 63 PFU/cell. *E. coli* phages (SU57) isolated from wastewater by Mirzaei and Nilsson (22) showed a latency of 17 mins, an uptake of 90% in 5 mins and a burst size of 196 PFU/cell. These differences in virulence data probably indicate that the phages isolated in the two studies are genetically different.

Several *in vitro* experimental models have described the effect of phages isolated from wastewater on the evolution of the population of associated pathogens (15,24). In our study, the increase in OD in the absence of BBec suspension (B) reflects the normal growth of the *E. coli* 1642 population in the broth, whereas the decrease in OD in the presence of BBec suspension (B+P) indicates the disruption of the normal growth of *E. coli* 1642. These observations can be explained by the fact that the phages in the BBec suspension infected the *E. coli* 1642 population causing massive lysis, hence the observed OD regression. Previous studies have made similar observations, for example, in the *in vitro* experiments by Wang et al., (17), MDR *E. coli* and methicillin-resistant *S. aureus* phages isolated from wastewater exhibited antibacterial activities against their respective populations.

Conclusion:

In our study, phage from clinical isolates of *E. coli* and *S. aureus* with reduced host range were isolated from random wastewater samples of urban sewage, lakes and lagoons in the city of Lomé, Togo. Demonstrating the virulence of one of the phage suspensions, designated BBec, allowed the determination of the latency of the phage, number of phages released per bacterial cell and degree of phage uptake. In addition, data demonstrating an antibacterial effect of the BBec suspension on the *E. coli* 1642 isolate was obtained by *in vitro* demonstration of the effect of the BBec suspension on the *E. coli* 1642 isolate.

Therefore, we can confirm that BBec suspension phages could be candidates for the development of new antibacterial therapies based on the scientific data obtained in this study. It would be interesting to extend the sampling sites to search for other phages specific to MDR bacterial strains, their characterization, study of the physical and chemical influences on their evolution, and *in vivo* experiments to better understand their application in therapeutics.

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Contributions of authors:

OAK conceived the study, collected the sample, participated in the experiments and analysis of the data, and drafted the manuscript; HY conceived the study, participated in its design, supervised the laboratory experiments, participated in the writing and corresponding author of the manuscript; GHE and PP participated in the experiments and analysis of the data; KK and SK participated in the sample collection, and helped to draft the manuscript; MM and DB were involved in the coordination of the study and corrected the manuscript; TT participated in the design of the study and supervised the work. All authors read and approved the final manuscript.

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Conflict of interest:

No conflict of interest is declared.

References:

1. Bouyahya, A., Bakri, Y., Et-Touys, A., et al. Résistance aux antibiotiques et mécanismes d'action des huiles essentielles contre les bactéries. Phytothérapie. 2018; 16 (S1): S173 - S183. doi: 10.1007/s10298-017-1118-z.
2. Chaudhary, A., S. A review of global initiatives to fight antibiotic resistance and recent antibiotics discovery. Acta Pharmaceutica Sinica B. 2016; 6(6): 552-6. doi: 10.1016/j.apsb.2016.06.004.
3. O' Neill, J. Review on Antimicrobial Resistance. Antimicrobial Resistance: Tackling a Crisis for the Health and Wealth of Nations. 2014.
4. Murray, C. J., Ikuta, K. S., Sharara., et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. The Lancet. 2022; 399(10325):629-655. doi: 10.1016/S0140-6736(21)02724-0.
5. Ouedraogo, A. S., Pierre, H. J., Bañuls, A. L., Ouédraogo, R., and Godreuil, S. Emergence and spread of antibiotic resistance in West Africa: contributing factors and threat assessment. Médecine et Santé Tropicales. 2017; 27 (2): 147-154. doi: 10.1684/mst.2017.0678.
6. Macrì F. Homéopathie et antibiorésistance. La Revue d'Homéopathie. 2019; 10(4): 162-165. doi: 10.1016/j.revhom.2019.10.018.
7. Dufour, N., and Debarbieux, L. La phagothérapie -Une arme crédible face à l'antibiorésistance. Med Sci (Paris). 2017; 33(4): 410-416. doi:10.1051/medsci/20173304011.
8. Ravat, F., Jault, P., and Gabard, J. Bactériophages et phagothérapie: utilisation de virus naturels pour traiter les infections bactériennes. Ann Burns Fire Disasters. 2015; 28 (1): 13-20.
9. Patey, O. La phagothérapie : des virus pour combattre les infections. In: Dublanchet, A. (Ed) Favre, Lausanne (Suisse), Hegel. 2017; 2(2):171-172.doi:10.4267/2042/62299.
10. Pirnay, J. P., Verbeken, G., Rose, T., et al. Introducing yesterday's phage therapy in today's medicine. Future Virol. 2012; 7 (4): 379-390. doi: 10.2217/fvl.12.24
11. Ansaldi, M., Boulanger, P., Brives, C., et al. Les applications antibactériennes des bactériophages Virologie. 2020; 24 (1): 23-36. doi: 10.1684/vir.2019.0805
12. Jordan, B. Cent ans après, le retour de la phagothérapie ? - Chroniques génomiques. Med Sci (Paris). 2019; 35 (10): 806-809. doi: 10.1051/medsci/2019149
13. Hoetzinger, M., Nilsson, E., Arabi, R., et al. Dynamics of Baltic Sea phages driven by environmental changes. Environ Microbiol. 2021; 23(8): 4576-94. doi: 10.1111/1462-2920.15651
14. Manohar, P., Tamhankar, A. J., Lundborg, C. S., and Ramesh N. Isolation, characterization and in vivo efficacy of *Escherichia* phage myPSH1131. PLoS One. 2018; 13 (10): e0206278. doi: 10.1371/journal.pone.0206278
15. Koudou, A. A., Kakou-Ngazoa, S., Addablah, A., et al. Biocontrôle de l'infection à *Pseudomonas aeruginosa* multi-résistant par les bactériophages en aquaculture en Côte d'Ivoire. J Appl Biosci. 2020; 154: 15940-9. doi: 10.35759/JABs.154.10
16. Magin, V., Garrec, N., and Andrés, Y. Selection of bacteriophages to control *in vitro* 24 h old biofilm of *Pseudomonas aeruginosa* isolated from drinking and thermal water. Viruses. 2019; 11 (8): 749. doi: 10.3390/v11080749
17. Wang, Z., Zheng, P., Ji, W., et al. SLPW: A Virulent Bacteriophage Targeting Methicillin-Resistant *Staphylococcus aureus In vitro* and *In vivo*. Front Microbiol. 2016. 7: 934. doi: 10.3389/fmicb.2016.00934
18. Ngazoa-Kakou, S., Philippe, C., Tremblay, D. M., et al. Complete genome sequence of Ebrios, a novel T7 virus isolated from the Ebrie Lagoon in Abidjan, Côte d'Ivoire. Genome Announcements. 2018; 6(25): e00280-18. doi: 10.1128/genomea.00280-18
19. Addablah, A. A., Kakou-Ngazoa, S., Akpa, E. E., et al. RAPD-based evaluation revealed genetically diverse populations of *Pseudomonas aeruginosa* and *Staphylococcus aureus* lytic bacteriophages isolated in urban sewage and Ebrie Lagoon, Côte d'Ivoire. AJMR. 2021; 15(10): 522-8. doi: 10.5897/AJMR2021.9547
20. Son, B., Yun, J., Lim J. A., et al. Characterization of LysB4, an endolysin from the *Bacillus cereus*-infecting bacteriophage B4. BMC Microbiol. 2012; 12 (1): 1-9.
21. Koskella, B., and Meaden, S. Understanding bacteriophage specificity in natural microbial communities. Viruses. 2013; 5(3): 806-823. doi; 10.3390/v5030806
22. Mirzaei, M. K., and Nilsson, A. S. Isolation of Phages for Phage Therapy: A Comparison of Spot Tests and Efficiency of Plating Analyses for Determination of Host Range and Efficacy. PLoS One. 2015; 10(3): e0118557. doi: 10.1371/journal.pone.0118557
23. Labrie, S. J., Samson, J. E., and Moineau, S. Bacteriophage resistance mechanisms. Nat Rev Microbiol. 2010; 8(5): 317-27.
24. Plaza, N., Castillo, D., Pérez-Reytor, D., et al. Bacteriophages in the control of pathogenic Vibrios. Electr J Biotechnol.2018; 31: 24-33. doi: 10.1016/j.ejbt.2017.10.012