Probiotic–Ceramic Viability For Antimicrobial Resistance Prevention

Temperature-Dependent Survival Analysis of *Bacillus subtilis* on Two Ceramic Materials Over 28 Days

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1. INTRODUCTION

1.1. Antimicrobial Resistance

AntiMicrobial resistance (AMR) is microorganisms' ability to survive previously effective antimicrobial exposure (Ahmed et al., 2024; Ferrara et al., 2024) - including antibiotics, antivirals, antifungals and antiparasitics (World Health Organization, 2023). AMR prevalence contributes to complications during treatment of infectious diseases and increases risk for operations (Catalano et al., 2022; Ferrara et al., 2024). The World Health Organization (WHO) identifies AMR as one of the greatest global public health threats of this century (Petersen et al., 2023). It is associated with over 4.71 million deaths worldwide in 2021, with projections estimating 39 million by 2050 if no effective intervention is undertaken (World Health Organization, 2024; The Lancet, 2024).

AMR is also a substantial economic burden. Global AMR-related healthcare costs and productivity losses are projected to cumulatively exceed US$100 trillion by 2050 (World Health Organization, 2024), highlighting the need for alternative approaches that reduce the emergence and transmission of resistant microorganisms.

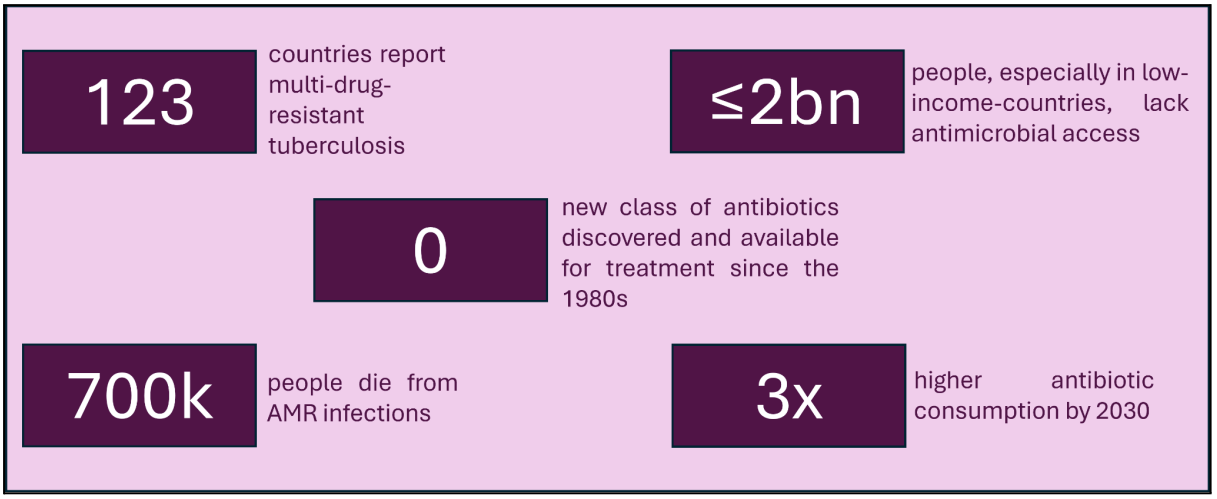

Figure 1. Key statistics illustrating the burden of antimicrobial resistance and underlining the urgency of finding AMR solutions. Data compiled from Dheda et al. (2017), O'Neill (2014), Silver (2011) and Klein et al. (2018)

Although AMR emergence is a natural evolutionary process caused by genetic mutation and horizontal gene transfer (Ferrara et al., 2024), it has accelerated due to

anthropogenic activity since the introduction of antibiotics in the 1940s (Ahmed et al., 2024). Antimicrobial misuse in human medicine, veterinary medicine and agriculture, alongside poor sanitation and the circulation of substandard medicines, has increased selective pressure for resistant organisms (Ahmed et al., 2024; Petersen et al., 2023), and thus AMR. Furthermore, declining antimicrobial research and development has limited the availability of new treatments, with many pharmaceutical companies withdrawing from antibiotic discovery due to high costs and limited financial incentives (Ferrara et al., 2024; Kayambankadzanja et al., 2020). Therefore, increasing attention has shifted towards preventative strategies that limit transmission rather than relying solely on new antimicrobial development.

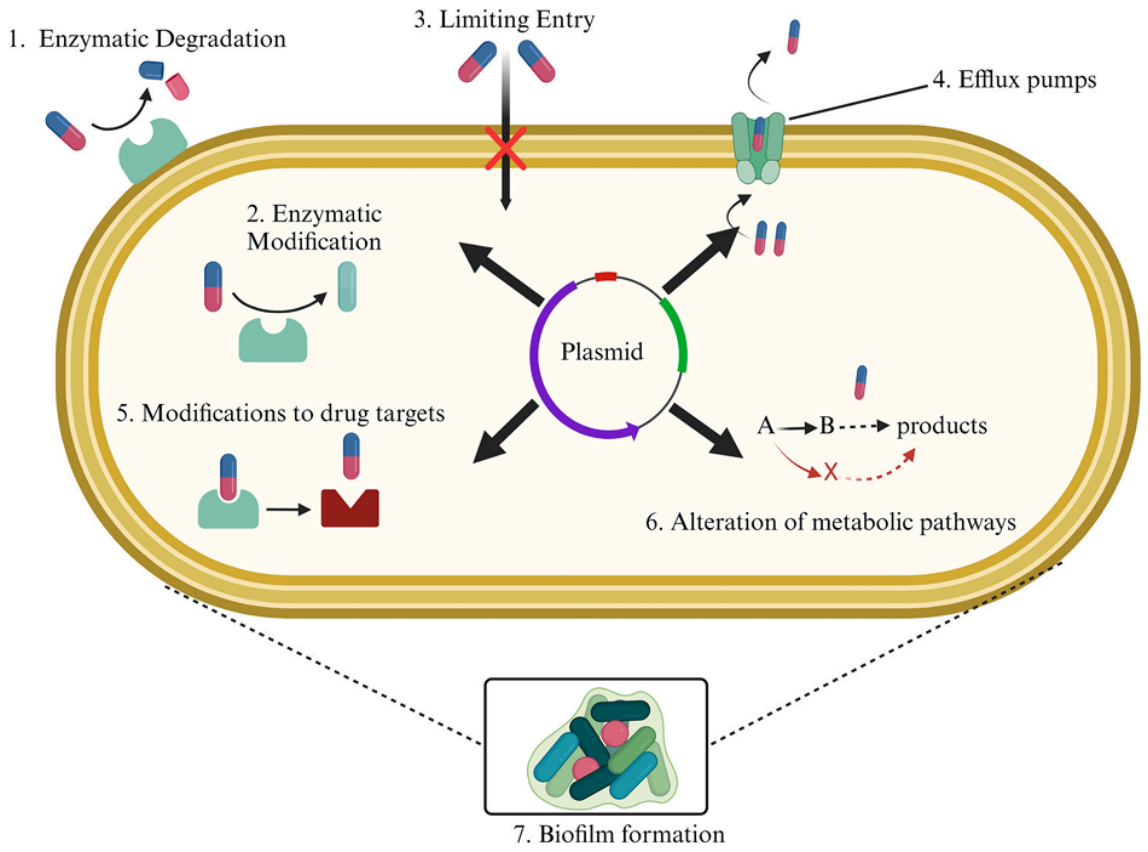

Figure 2. Mechanisms of AMR in bacteria. (1) enzymes break down antimicrobial agents, inactivating them (2) antimicrobial agents chemically altered and inactivated; (3) reduced membrane permeability prevents antimicrobial agents from entering the cell; (4) expulsion of antimicrobial agents from the cell; (5) reduces antimicrobial binding or activity; (6) enables bacteria to bypass antimicrobial effects; (7) provides a protective environment that reduces antimicrobial penetration and increases bacterial tolerance. Reproduced from (Ahmed et al.,

1.2. AMR in the Built Environment

The built environment represents an important reservoir for AMR microorganisms. Indeed, indoor and frequently touched surfaces support pathogen persistence and contribute to transmission **(Figure 3)**. Although healthcare workers' hands remain the primary route of transmission in hospitals, patients can contaminate surrounding surfaces with clinically relevant pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and *Clostridioides difficile*, which are major causes of healthcare-associated infections and persist despite routine disinfection (Otter, Yezli and French, 2011; Wißmann et al., 2021).

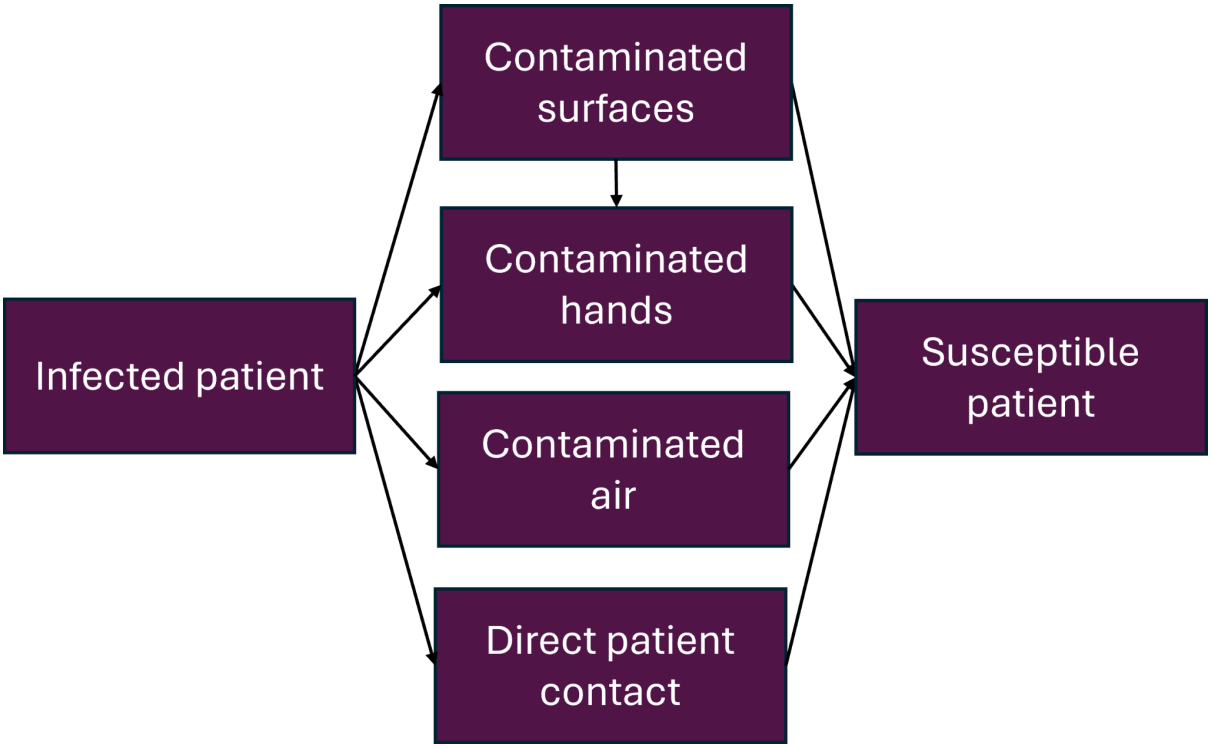

Figure 3. Main transmission routes of AMR pathogens. Author-created schematic based on Otter, Yezli and French (2011)

This infection-control challenge extends beyond healthcare settings, as fomite-mediated transmission has been implicated in community outbreaks and surface contamination still represents a transmission risk (Otter, Yezli and French, 2011; Wißmann et al., 2021). As humans spend most of their time indoors, building materials act as persistent microbial reservoirs, particularly where moisture supports microbial survival and growth (Jabłońska-Trypuć et al., 2022; Mhuireach et al., 2021) . Current infection-control approaches rely heavily on chemical disinfectants; yet, these can fail to prevent recolonisation and may contribute to resistant microorganisms selection (Caselli, 2017).

1.3. Probiotic Building Materials

However, emerging research challenges assumptions that healthier buildings contain fewer microorganisms. Instead, there is evidence that microbial communities can contribute to human health via immune development and ecological stability (Mhuireach et al., 2021; Beckett, 2023). The NOTBAD (Niches for Organic Territories in Bio-Augmented Design) (UKRI, 2021) project, developed by Nair and Beckett at University College London (UCL), explored this concept by the incorporation of microorganisms into building materials to prevent AMR pathogen colonisation. Unlike “kill-all” strategies, NOTBAD established microbial communities that reduce the spread of harmful microorganisms and survival rates. The project concluded that “probiotic microbes integrated into bio-receptive ceramic materials can survive and outcompete harmful microbes under laboratory conditions”. Further research at the UCL Eastman Dental Institute concluded that at least two ceramic materials - ceramic X and ceramic Z - allowed for *B. subtilis* survival the most.

Probiotic Cleaning Hygiene Systems (PCHS), which use three *Bacillus* species to establish beneficial surface microbiota, support the findings of the NOTBAD project. Caselli (2017) showed that PCHS reduced surface pathogens by approximately 90% compared with conventional disinfectants while decreasing AMR gene survival. Among PCHS bacteria, *Bacillus subtilis* is particularly promising as it forms resilient endospores capable of surviving harsh environmental conditions (Piewngam et al., 2018). Additionally, *B. subtilis* inhibits pathogen colonisation via Agr quorum sensing interference, reducing pathogen establishment - all whilst leaving the wider microbial community undisrupted (Piewngam et al., 2018).

1.4. Temperature-Dependent Survival of *B. subtilis*

Despite evidence supporting probiotic approaches, there is limited comprehension regarding environmental conditions' influence on probiotic survival, once incorporated into ceramics. In fact, temperature may affect *B. subtilis* survival and therefore influence its ability to establish and suppress pathogen colonisation. This is significant in real-world applications as internal building temperatures vary significantly due to seasons, time of day and occupant behaviour, among other factors.

Survival might follow the trend of *B. subtilis* growth and increase with temperature. Hoffmann and Bremer (2011) reported a minimal growth temperature of 15°C at 0.03 h^{-1}. This is due to *B. subtilis*' enzyme-driven metabolism, with lower temperatures decreasing enzyme activity, glycolysis, ATP production, DNA replication and protein synthesis (Budde et al., 2006). These reduce bacterial growth rate. In parallel, cold causes membrane fluidity reduction, impairing nutrient transport, resulting in slower growth rates. The opposite occurs with an increase in temperature. Thus, this might suggest that *B. subtilis* survival might also increase with temperature, just like growth does, as it is more optimal for the cellular functions of the bacteria. However, this relationship may not necessarily translate into large differences in survival across the temperatures tested. *B. subtilis* is a soil bacterium adapted to fluctuating environmental conditions, and its ability to persist under relatively suboptimal temperatures may reduce the extent of temperature-dependent differences in long-term survival. Furthermore, while higher temperatures may favour more rapid growth, they may not necessarily maximise long-term survival. Increased bacterial cell survival can accelerate nutrient depletion in a limited-resource environment, potentially reducing survival over extended periods.

1.5. Research Rationale and Aim

This study investigates the influence of temperature on *Bacillus subtilis* survival in ceramic materials, allowing for evaluation of probiotic ceramics as a preventative strategy against AMR pathogen transmission.

2. METHODOLOGY

2.1. Growth of Bacterial Species

10 mL nutrient broth was inoculated with a single colony of *B. subtilis* and incubated for 18 h at 30°C. OD600 was measured and adjusted to 10.0.

2.2. Ceramic Materials

Ceramics were selected as they are commonplace, relatively inexpensive and are easy to fabricate with - including casting and 3D printing. This means that they are adaptable and can be applied within a wide range of building types, including architectural features or design elements.

Two ceramic disc materials - X and Z - were tested as potential probiotic survival surfaces, as when seeded with *B. subtilis* they had the highest levels of inhibitory activity against MRSA after 28 days in previous studies by W. Scott (UCL).

2.3. Survival of *B. subtilis* at Different Temperatures

Ceramic discs of both materials were inoculated: 18 discs per ceramic material were inoculated with 200 µL *B. subtilis* culture, with 9 discs per ceramic material inoculated with 200 µL nutrient broth as controls. Discs were then left at room temperature to absorb *B. subtilis* for 60 minutes. Discs were then removed and placed into sterile petri dishes. 6 inoculated discs per material per temperature were incubated at three different temperatures - 16°C, 21°C and 30°C - with 3 control discs per material being incubated at each temperature. The former temperature is the Health and Safety Executive minimum suggested working temperature, while 21°C is the standard indoor temperature, and the latter temperature represents extreme indoor temperatures which may occur in some parts of the UK. Two inoculated discs per material per temperature were removed at 3 chosen timepoints - Day 1, Day 5, Day 28 - alongside one control per material per temperature. The first two timepoints were selected to assess initial bacterial survival, while Day 28 was selected to assess

long-term survival, as previous trials demonstrated inhibitory activity against MRSA after 28 days.

After the test time elapsed, discs were vortexed for 60 seconds in 10 mL PBS-filled tubes. 0.1 mL of each tube solution was added to 0.9 mL Mueller Hinton Broth-filled Eppendorf tubes. After vortexing, 50 µL of the solution was added to separate wells in a 96-well plate, each also filled with 150 µL of MHB. A breathable membrane was placed over the 96-well plate and then an OD600 was measured every 15 minutes for 20h at 30°C.

Table 1. Experimental setup for this study

	Day 1	Day 5	Day 28	
Ceramic X	2 incubated + 1 control	2 incubated + 1 control	2 incubated + 1 control	16°C
Ceramic Z	2 incubated + 1 control	2 incubated + 1 control	2 incubated + 1 control	
Ceramic X	2 incubated + 1 control	2 incubated + 1 control	2 incubated + 1 control	21°C
Ceramic Z	2 incubated + 1 control	2 incubated + 1 control	2 incubated + 1 control	
Ceramic X	2 incubated + 1 control	2 incubated + 1 control	2 incubated + 1 control	30°C
Ceramic Z	2 incubated + 1 control	2 incubated + 1 control	2 incubated + 1 control	

2.4. Data Analysis

All samples were analysed by the SPECTROstar Nano microplate reader (BMG LABTECH, Germany) and collected. Data was analysed in RStudio to calculate the Midpoint time (t_mid) value of each sample by using the "growthcurver" package in RStudio. All controls showed no bacterial survival.

t_mid values were then inserted into a regression equation (established by W. Scott, UCL) to estimate the colony-forming units (CFU)/plate for each sample. For *B. subtilis*, the relationship between t_mid and cell count is described by

$t\_mid = 11.3 - 1.4\, log_{10}(cell\ count)$. This formula was rearranged to calculate cell count as: $cell\ count = \frac{10(t\_mid - 11.3)}{-1.4}$. Cell count represents the estimated cell count and t_mid represents the midpoint time obtained from the growth-curve analysis. During RStudio analysis, negative t_mid values were classified as indicating no detectable survival. Samples with low t_mid values were subsequently assessed by inspecting the corresponding growth curves to determine whether the observed signal represented genuine bacterial survival. The resulting cell counts were used as the CFU/plate values for subsequent analysis. Said values were log_{10}-transformed for ease of interpretation **(Appendix I** and **Appendix II)**. These values' means were then calculated and graphed. **(Table 2** and **3) (Figure 5** and **6)**.

3. RESULTS

This was a pilot study and as such no statistical analysis was possible due to the low N number in the experiment.

3.1. Processed Data

Table 2. Processed data table with average estimated log_{10}(CFU) values of *B. subtilis* with Ceramic X

Average estimated log10(CFU) of *B. subtilis* on Ceramic X			
Temperature	Day		
	1	5	28
16°C	5.79	2.77	0.00
21°C	7.18	4.72	0.00
30°C	6.30	6.16	6.04

Table 3. Processed data table with average estimated log_{10}(CFU) values of *B. subtilis* with Ceramic Z

log10(CFU) of *B. subtilis* on Ceramic Z			
Temperature	Day		
	1	5	28
16°C	4.72	4.13	0.00
21°C	7.29	6.16	2.18
30°C	6.32	5.91	5.56

3.2. Analysis And Results For Ceramic X

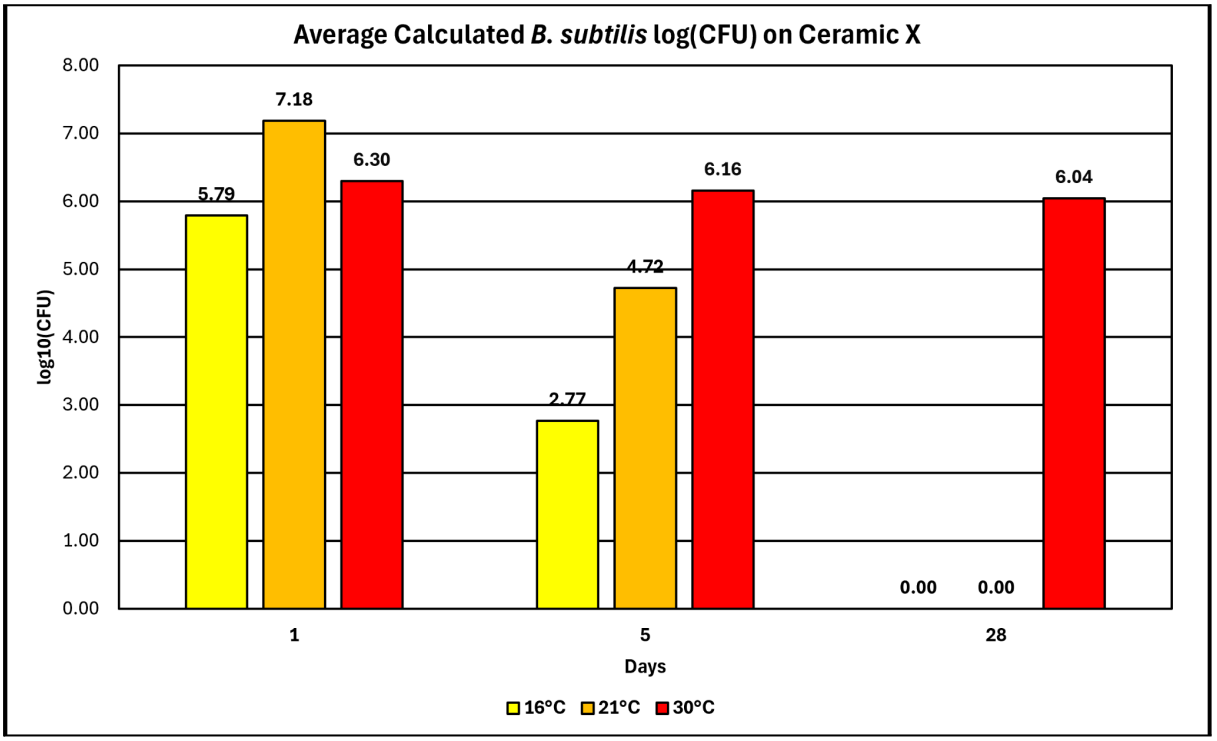

Fig 5. Graph of the average estimated log_{10}(CFU) values for ceramic X

- Day 1: Bacterial survival was higher at 21°C than at either 16°C or 30°C, while CFU values at 16°C and 30°C were similar.
- Day 5: Bacterial survival increased across the temperature groups in the order 16°C < 21°C < 30°C, with each temperature showing a different CFU value.
- Day 28: Bacterial survival was highest at 30°C, while no viable bacteria were detected at either 16°C or 21°C ($CFU = 0$ for both temperatures)

3.3. Analysis And Results For Ceramic Z

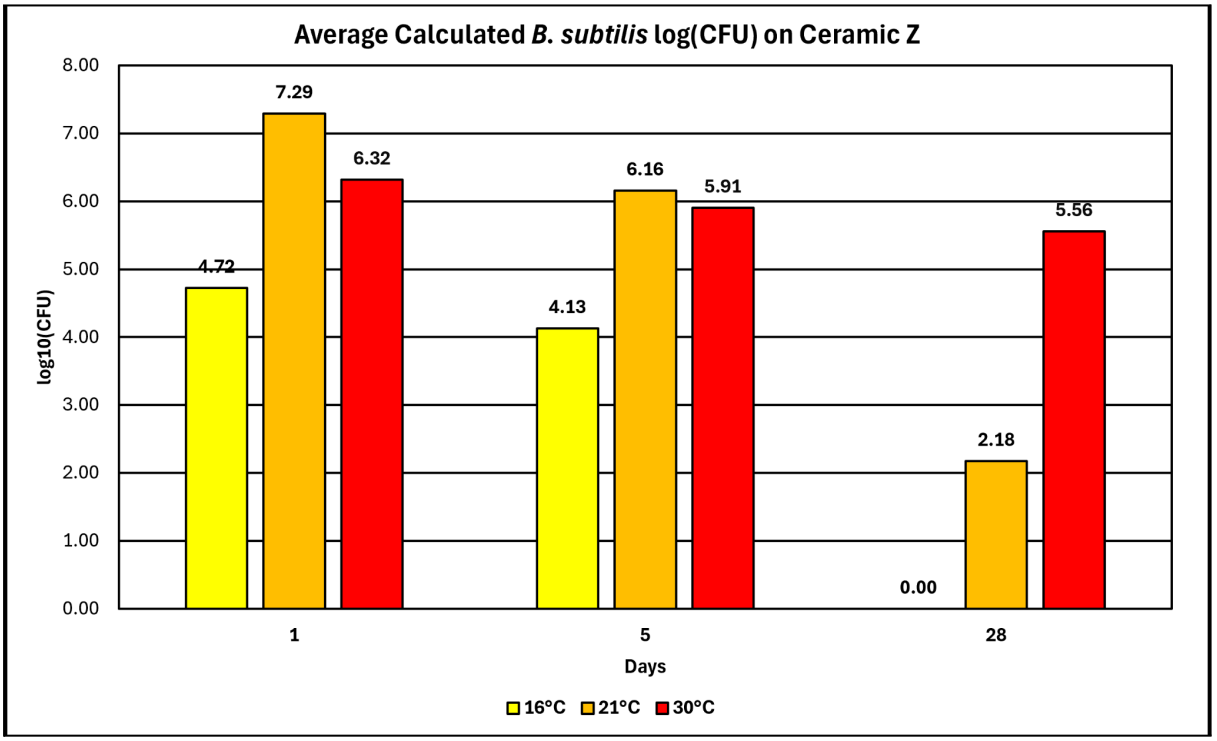

Fig 7. Graph of the average estimated log_{10}(CFU) values for ceramic Z

- Day 1: Bacterial survival was higher at 21°C than at either 16°C or 30°C, while 16°C had the lowest CFU value.
- Day 5: Bacterial survival was higher at both 21°C and 30°C than at 16°C, while CFU values at 21°C and 30°C were similar. Overall, bacterial survival was lower at 16°C than at the two higher temperatures.
- Day 28: Bacterial survival was highest at 30°C, while no viable bacteria were detected at either 16°C (CFU = 0). 21°C was between the two other temperatures.

4. DISCUSSION

4.1. Temperature-Dependent Survival at 16°C

16°C produced the lowest survival at all timepoints - except when no detectable recoverable CFU also occurred at 21°C on Day 28 - with values of 5.79, 2.77 and 0 for ceramic X, and 4.72, 4.13 and 0 for ceramic Z. This is consistent with the negative effect 16°C had in other studies (Neale and Chapman, 1970; Beckering et al., 2002; Graumann et al., 1996; Weber et al., 2001; Baruah et al., 2026; Fan et al., 2025) due to physiological stress imposed at this temperature. Yet, low temperatures do not necessarily eliminate the population directly from Day 1; some cells can remain viable and functionally active despite markedly reduced growth (Brigulla et al., 2003; Neale and Chapman, 1970; Beckering et al., 2002; Graumann et al., 1996; Weber and Marahiel, 2002; Weber et al., 2001; Wiegeshoff et al., 2006; Baruah et al., 2026; Fan et al., 2025). This is consistent with the progressive decrease in bacterial survival observed at 16°C across the timepoints in both materials, rather than sudden decrease with immediate elimination.

This physiological stress involves reduced metabolic activity and growth, altered membrane properties, disrupted translation and cell-envelope processes, and activation of stress-adaptation systems (Brigulla et al., 2003; Neale and Chapman, 1970; Kaan et al., 2002; Beckering et al., 2002; Graumann et al., 1996; Weber and Marahiel, 2002; Weber et al., 2001; Wiegeshoff et al., 2006; Baruah et al., 2026; Fan et al., 2025). Specifically, metabolism across glycolysis, the TCA cycle, ATP synthesis and biosynthetic pathways is reduced, while protein synthesis is altered (Beckering et al., 2002; Graumann et al., 1996). Thus, 16°C can place *B. subtilis* in a physiologically constrained state in which growth is reduced while adaptive responses are activated. However, these studies predominantly examined cells in liquid culture, rather than cells associated with a ceramic surface as in the present study. Consequently, their findings provide physiological context for interpreting the 16°C condition but should not be assumed to directly predict survival on ceramic surfaces. To our knowledge, comparable studies examining long-term *B. subtilis* survival on ceramic surfaces across these temperatures are limited, necessitating comparison with the available liquid-culture literature. Studies demonstrating persistence of *B. subtilis* at temperatures within this range, including 15–18°C (Neale and Chapman, 1970; Beckering et al., 2002; Graumann et al., 1996; Weber et al., 2001; Fan et al., 2025), therefore provide supporting

rather than directly comparable evidence for the survival observed on Day 1 in the present study.

However, this constrained state affected the bacteria differently between the ceramics during the early stages. Although both showed decreased survival overall, ceramic X declined nearly four times more than ceramic Z by Day 5, from 5.79 to 2.77 and from 4.72 to 4.13 respectively. Thus, ceramic X showed a greater rate of survival decline than ceramic Z initially. Yet, both ceramics reached Day 28 with no detectable recoverable CFU, demonstrating that long-term outcomes were ultimately similar despite initially differing trajectories. Since the same *B. subtilis* strain was used throughout, despite known strain-specific differences in cold adaptation (Fan et al., 2025), this initial divergence may instead reflect differences between the ceramic microenvironments.

Differences in ceramic porosity and water retention could generate distinct conditions for bacterial survival. Water retention in porous ceramic depends on capillary and adsorptive forces, including surface geometry and roughness (Dechesne et al., 2008), while lower matric potentials reduce aqueous-film thickness and connectivity, restricting bacterial movement and confining cells to isolated water pools (Tecon and Or, 2016). If ceramic X retains less moisture than ceramic Z, reduced water availability could therefore impose greater water stress (Dechesne et al., 2008; Tecon and Or, 2016). Ceramic pore architecture also influences water transport and bacterial survival: micropores facilitate capillary transport, while larger pores provide space and reservoirs for bacterial survival (Dutto et al., 2024). More porous ceramics have been shown to support greater bacterial infiltration and proliferation, with hierarchical macro-microporous structures producing the greatest biomass, demonstrating that pore architecture - not simply total porosity - can influence bacterial biomass (Dutto et al., 2024). Bacterial activity can additionally modify the local microenvironment through the production and excretion of compounds (Dutto et al., 2024).

Therefore, if ceramic X retains less moisture, the resulting reduction in water availability may have imposed greater environmental stress on the incorporated cells. This could have influenced stress-adaptation pathways, including those associated with membrane adaptation, while prolonged stress may also have contributed to cell-wall damage and loss of viability (Baruah et al., 2026). Thus, differences in ceramic microenvironments may explain why the rate of bacterial survival decline differed between the two ceramics, even though both ultimately reached no detectable recoverable CFU by Day 28.

4.2. Temperature-Survival at 21°C

Both ceramics demonstrated a decrease in recoverable bacteria over the timepoints at 21°C, with ceramic X showing a greater decline than ceramic Z. Between Days 1 and 5, ceramic X declined from 7.18 to 4.72 CFU, whereas ceramic Z declined from 7.29 to 6.16 CFU, meaning that the decline on ceramic X was more than twice that observed on ceramic Z. Ceramic X subsequently continued to decline at a greater rate, ultimately reaching no detectable recoverable CFU by Day 28, whereas ceramic Z retained 2.18 CFU. Notably, 21°C was the only temperature at which the two ceramics showed a substantial difference in CFU at Day 28. This ability of *B. subtilis* to persist up to Day 28 on ceramic Z is consistent with previous findings at the Eastman Dental Institute.

As highlighted in Section 4.1, differences in ceramic microenvironments might explain the difference in CFU observed at Day 28, with moisture availability playing a substantial role in long-term *B. subtilis* survival. Despite both ceramics displaying the highest recorded CFUs on Day 1, ceramic X reached the same endpoint observed at 16°C, with no detectable recoverable CFU, while ceramic Z retained detectable bacteria at Day 28.

This Day 1 finding is not abnormal as Méndez et al. (2004) demonstrated that *B. subtilis* can remain viable during exposure to 20°C, with σB and Spo0A contributing to survival. However, their study investigated bacteria in liquid culture and measured viability using CFU, whereas the present study assessed subsequent survival following recovery from ceramic surfaces. Therefore, the persistence observed on ceramic Z may reflect not only the ability of *B. subtilis* to survive at temperatures around 20°C, but also the specific microenvironment provided by the ceramic surface.

Another possible explanation, beyond simply providing a more favourable microenvironment for survival, is that ceramic Z may instead provide a more stressful microenvironment that influences pathways also involved in cold-shock responses, potentially promoting bacterial survival. In fact, Fan et al. (2025) found that *B. subtilis* failed to grow over two days at 15°C but adapted and resumed exponential growth at 18°C in a nutrient-rich medium. This illustrates the ability of *B. subtilis* to undergo physiological adaptation in response to low temperature. Importantly, cold-shock responses are primarily induced by low temperatures rather than nutrient limitation; however, the nutrient-limited conditions of the ceramic surface may have imposed an additional stress that influenced

bacterial persistence and stress adaptation. Membrane adaptation is a major mechanism supporting persistence under low-temperature and stressful conditions (Beckering et al., 2002; Weber and Marahiel, 2002; Weber et al., 2001; Wiegeshoff et al., 2006). Low temperature alters membrane properties, prompting changes in fatty-acid composition to maintain membrane function (Weber and Marahiel, 2002; Weber et al., 2001). The Des fatty-acid desaturase is important for this response, with disruption of the Des pathway producing a strong cold-sensitive phenotype at 15°C; its activity promotes the production of unsaturated fatty acids that compensate for membrane rigidification (Weber et al., 2001). The σL/BkdR pathway can similarly contribute through branched-chain fatty-acid metabolism and membrane adaptation (Wiegeshoff et al., 2006). Low temperature also alters ribosomal and translation-associated functions, indicating continued cellular reorganisation rather than complete metabolic shutdown (Kaan et al., 2002; Beckering et al., 2002; Graumann et al., 1996). Thus, at 21°C, the combination of relatively low temperature and the low-resource conditions of the ceramic surface may have required continued stress adaptation. It is therefore possible that ceramic Z provided a microenvironment in which a proportion of the bacterial population maintained such adaptive responses, potentially contributing to its persistence through Day 28.

4.3. Temperature-Dependent Survival at 30°C

Survival patterns at 30°C differed greatly from the fluctuations observed at the lower temperatures, demonstrating relatively stable persistence throughout the timepoints of this study. Ceramic X decreased only slightly from 6.30 CFU on Day 1 to 6.04 CFU on Day 28, while ceramic Z decreased from 6.32 to 5.56 CFU. This survival is consistent with the favourable conditions that 30°C provides to *B. subtilis*. Hoffmann and Bremer (2011) noted a steady increase in CFU count as environmental temperature increased, up to 35°C. This might be due to what Budde et al. (2006) describe as processes associated with warmer conditions, including sustained glycolysis, protein synthesis and enzyme activity, contrasting with the cold-shock responses induced at lower temperatures. Thus, 30°C may have permitted the bacteria to maintain metabolic activity throughout the study's timepoints.

Moreover, Adhitya Pitara Sanjaya et al. (2023) reported *B. subtilis* growth at this temperature with a CFU value of 7.83 after 1 day, compared with 6.30 and 6.32 CFU for

ceramic X and ceramic Z respectively in the present study. The difference in CFU values may reflect the more favourable conditions in their study, where bacteria were cultured in nutrient-rich liquid medium, compared with the nutrient-limited, surface-associated conditions of the present study on ceramic discs. Similarly, Uju Marie-Esther (2014) demonstrated that *B. subtilis* can undergo substantial growth at 30°C before transitioning to a stationary phase during prolonged incubation. Their bacteria reached a peak CFU after 3 days, followed by a prolonged stationary phase and subsequent decline, demonstrating that a favourable temperature does not necessarily result in continuous population increase during extended incubation. This is similar to the relatively stable CFU values observed on the ceramics in the present study, although their liquid-culture conditions are not directly comparable with the surface-associated environment examined here.

On the other hand, Sebiomo (2020) found that CFU increased during the initial period of incubation, reaching a maximum at approximately 10 days before subsequently declining. These findings similarly suggest that although 30°C supports *B. subtilis* survival, prolonged incubation can eventually reduce bacterial viability. However, Sebiomo (2020) used continuously shaken liquid cultures containing nutrients and herbicides, whereas the present study examined bacteria persisting on ceramic surfaces. The nutrient-limited conditions of the present study may therefore have constrained bacterial proliferation and prevented a substantial increase in recoverable CFU, as explained in 1.4. Once available resources became insufficient to sustain the population, this may instead have resulted in a gradual decline in recoverable bacteria. This could also explain the difference in the timing of peak CFU between Uju Marie-Esther (2014), where peak CFU occurred after 3 days, and the present study, where no comparable increase was observed.

Nevertheless, these studies support the interpretation that 30°C is compatible with prolonged *B. subtilis* persistence without necessarily producing continuous proliferation throughout extended incubation. The relatively small decline observed on both ceramics therefore suggests that, under the nutrient-limited conditions of the present study, 30°C provided a comparatively favourable temperature for maintaining recoverable bacteria over 28 days.

4.4. Overall Comparison of *B. subtilis* Survival on Different Ceramics And Temperatures

Overall, temperature appeared to have a greater influence on *B. subtilis* survival than ceramic type. Survival declined most substantially at 16°C, where both ceramics reached no detectable recoverable CFU by Day 28, whereas 30°C supported comparatively stable survival on both ceramics. The 21°C condition produced an intermediate pattern, although ceramic Z retained 2.18 CFU on Day 28 while ceramic X reached no detectable recoverable CFU.

Nevertheless, ceramic differences remained and were most apparent at 21°C and during the early stages at 16°C. In the latter situation, ceramic X declined more rapidly than ceramic Z between Days 1 and 5, although both ultimately reached the same endpoint. At 21°C, this difference persisted to Day 28, whereas at 30°C both ceramics showed relatively similar, stable survival. This may indicate that differences in ceramic microenvironment, particularly moisture availability and pore structure, become more consequential when environmental conditions impose greater stress on bacterial persistence (Dechesne et al., 2008; Tecon and Or, 2016; Dutto et al., 2024).

Thus, while temperature appeared to determine the overall survival trajectory, ceramic type appeared to modify the rate and extent of decline, particularly under the more stressful 16°C and 21°C conditions.

5. CONCLUSION

This pilot study investigated the survival of *B. subtilis* on ceramic X and ceramic Z ceramics at 16°C, 21°C and 30°C over 1, 5 and 28 days. This study suggests that although ceramic-specific differences were observed at certain temperatures and timepoints, temperature has a greater influence on survival than ceramic type.

These findings suggest that *B. subtilis* can persist on both ceramic materials across a range of temperatures, although the extent and rate of survival varied with temperature, incubation period and ceramic type. The results therefore provide preliminary evidence that

ceramic surfaces can support prolonged persistence of *B. subtilis* under certain environmental conditions, although further research is required to determine whether this persistence can be translated into an effective probiotic ceramic system and whether ceramic-specific differences are statistically significant.

6. LIMITATIONS AND FUTURE WORK

The limited sample size restricts the ability to do statistical analysis. With only two independently inoculated discs per condition, individual variation may disproportionately influence observed patterns.

Furthermore, regarding the cell-counting methodology, spores may have formed under stressful conditions (Higgins and Dworkin, 2012), particularly at 16°C, and subsequently been detected as viable cells during the growth-curve analysis. Consequently, the calculated CFU values may have overestimated the abundance of vegetative cells, particularly under prolonged or stressful incubation conditions. Nevertheless, substantial spore formation is unlikely to have occurred during the growth-curve analysis, as sporulation is generally favoured under nutrient-limited conditions, whereas the nutrient-rich medium used in the plate reader would instead support spore germination and subsequent outgrowth into vegetative cells.

The static nature of the temperatures utilised in this study make it different to real indoor environments where there is a variation in temperature within the day itself, occupant presence or season of year. Thus, this study may not allow us to understand how *B. subtilis* would respond in fluctuating temperatures more specifically.

Although the larger study by the UCL group aims to understand whether we can use probiotic ceramics as an AMR prevention strategy, my study has not examined whether surviving bacterial populations retained their inhibitory capacity against pathogens. In fact, my study assessed survival alone. Future work should build on this pilot study and include more replicates to allow statistical analysis. There must be confirmation of the CFU estimates via direct plate counts.

7. APPENDIX

Appendix I. Processed data table with estimated log_{10}(CFU) values of *B. subtilis* with ceramic X

Estimated log10(CFU) of *B. subtilis on Ceramic X*				
Temperature	Well #	Day		
		1	5	28
16°C	A2	6.22	2.76	0.00
	A3	5.37	2.78	0.00
21°C	A5	7.14	4.60	0.00
	A6	7.23	4.84	0.00
30°C	A8	6.31	6.06	6.10
	A9	6.28	6.26	5.99

Appendix II. Processed data table with estimated log_{10}(CFU) values of *B. subtilis* with ceramic Z

Temperature	Estimated log10(CFU) of *B. subtilis* on Ceramic Z			
	Well #	Day		
		1	5	28
16°C	B2	4.70	3.70	0.00
	B3	4.75	4.57	0.00
21°C	B5	7.30	6.09	3.45
	B6	7.29	6.23	0.91
30°C	B8	6.22	6.37	5.56
	B9	6.42	5.44	5.56

REFERENCE LIST

Adhitya Pitara Sanjaya, Danar Praseptiangga, Muhammad Zukhrufuz Zaman, Umiati, V.F. and Baraja, S.I. (2023). Effect of pH, temperature, and salt concentration on the growth of bacillus subtilis T9-05 isolated from fish sauce. *IOP conference series*, 1200(1), pp.012050–012050. doi:10.1088/1755-1315/1200/1/012050.

Ahmed, S.K., Husein, S., Qurbani, K., Ibrahim, R.H., Fareeq, A., Mahmood, K.A. and Mohamed, M.G. (2024). Antimicrobial resistance: Impacts, challenges, and future prospects. *Journal of Medicine Surgery and Public Health*, [online] 2(100081), pp.100081–100081. doi:https://doi.org/10.1016/j.glmedi.2024.100081.

Baruah, L., Sidarta, M., Hammer úr Skúoy, P., Johnsson, O., Frisk, E., Didelot, P., Arshad, A. and Wenzel, M. (2026). Contribution of the *bacillus subtilis ytrGABCDEF* operon to antibiotic stress adaptation. *Microbiology Spectrum*, 14(1). doi:10.1128/spectrum.02694-25.

Beckering, C.L., Steil, L., Weber, M.H.W., Völker, U. and Marahiel, M.A. (2002). Genomewide transcriptional analysis of the cold shock response in *bacillus subtilis*. *Journal of Bacteriology*, 184(22), pp.6395–6402. doi:10.1128/jb.184.22.6395-6402.2002.

Beckett, R. (2023). *Probiotic Cities*. Taylor & Francis.

Brigulla, M., Hoffmann, T., Krisp, A., Völker, A., Bremer, E. and Völker, U. (2003). Chill induction of the SigB-Dependent general stress response in*bacillus subtilis*and its contribution to low-temperature adaptation. *Journal of Bacteriology*, [online] 185(15), pp.4305–4314. doi:10.1128/jb.185.15.4305-4314.2003.

Budde, I., Scharf, C., Volker, U. and Bremer, E. (2006). Adaptation of Bacillus subtilis to growth at low temperature: A combined transcriptomic and proteomic appraisal. *Microbiology*, 152(3), pp.831–853. doi:10.1099/mic.0.28530-0.

Caselli, E. (2017). Hygiene: microbial strategies to reduce pathogens and drug resistance in clinical settings. *Microbial Biotechnology*, 10(5), pp.1079–1083. doi:10.1111/1751-7915.12755.

Catalano, A., Iacopetta, D., Ceramella, J., Scumaci, D., Giuzio, F., Saturnino, C., Aquaro, S., Rosano, C. and Sinicropi, M.S. (2022). Multidrug Resistance (MDR): A Widespread Phenomenon in Pharmacological Therapies. *Molecules*, [online] 27(3), p.616. doi:10.3390/molecules27030616.

CDC STACKS. (2016). *Making Health Care Safer: Protect Patients from Antibiotic Resistance: CDC Vital Signs: 03/03/2016*. [online] Available at: https://stacks.cdc.gov/view/cdc/38288 [Accessed 27 June 2026].

Cleveland Clinic (2024). *Methicillin-Resistant Staphylococcus Aureus (MRSA)*. [online] Cleveland Clinic. Available at: https://my.clevelandclinic.org/health/diseases/11633-methicillin-resistant-staphylococcus-aureus-mrsa.

Dechesne, A., Or, D., Gülez, G. and Smets, B.F. (2008). The porous surface model, a novel experimental system for online quantitative observation of microbial processes under unsaturated conditions. *Applied and environmental microbiology*, [online] 74(16), pp.5195–200. doi:10.1128/AEM.00313-08.

Department of Health and Social Care (2019). *UK 5-year action plan for antimicrobial resistance 2019 to 2024*. [online] GOV.UK. Available at: https://www.gov.uk/government/publications/uk-5-year-action-plan-for-antimicrobial-resistance-2019-to-2024 [Accessed 8 Sept. 2026].

Dheda, K., Gumbo, T., Maartens, G., Dooley, K.E., McNerney, R., Murray, M., Furin, J., Nardell, E.A., London, L., Lessem, E., Theron, G., van Helden, P., Niemann, S., Merker, M., Dowdy, D., Van Rie, A., Siu, G.K.H., Pasipanodya, J.G., Rodrigues, C., Clark, T.G., Sirgel, F.A., Esmail, A., Lin, H.-H., Atre, S.R., Schaaf, H.S., Chang, K.C., Lange, C., Nahid, P., Udwadia, Z.F., Horsburgh, C.R., Churchyard, G.J., Menzies, D., Hesseling, A.C., Nuermberger, E., McIlleron, H., Fennelly, K.P., Goemaere, E., Jaramillo, E., Low, M., Jara, C.M., Padayatchi, N. and Warren, R.M. (2017). The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis. *The Lancet Respiratory Medicine*, [online] 5(4), pp.291–360. doi:10.1016/S2213-2600(17)30079-6.

Dutto, A., Kan, A., Saraw, Z., Maillard, A., Zindel, D. and Studart, André R (2024). Living porous ceramics for bacteria-regulated gas sensing and carbon capture. *arXiv (Cornell University)*, [online] 37(5). doi:10.1002/adma.202412555.

Fan, H., Briercheck, A., Mutalik, V.K., Xiong, M. and Zhang, Y. (2025). Cross-species phenotypic profiling uncovers functional determinants of bacterial cold shock adaptation. *bioRxiv : the preprint server for biology*, [online] p.2025.09.22.676666. doi:10.1101/2025.09.22.676666.

Ferrara, F., Castagna, T., Pantolini, B., Campanardi, M.C., Roperti, M., Grotto, A., Fattori, M., Maso, L.D., Carrara, F., Giulia Zambarbieri, Zovi, A., Maurizio Capuozzo and Langella, R. (2024). The challenge of antimicrobial resistance (AMR): current status and future prospects. *Naunyn-Schmiedeberg s Archives of Pharmacology*, 397. doi:10.1007/s00210-024-03318-x.

Graumann, P., Schröder, K., Schmid, R. and Marahiel, M.A. (1996). Cold shock stress-induced proteins in bacillus subtilis. *Journal of Bacteriology*, 178(15), pp.4611–4619. doi:10.1128/jb.178.15.4611-4619.1996.

Higgins, D. and Dworkin, J. (2012). Recent progress in bacillus subtilis sporulation. *FEMS microbiology reviews*, [online] 36(1), pp.131–48. doi:10.1111/j.1574-6976.2011.00310.x.

Hoffmann, T. and Bremer, E. (2011). Protection of*Bacillus subtilis*against Cold Stress via Compatible-Solute Acquisition. *Journal of Bacteriology*, [online] 193(7), pp.1552–1562. doi:10.1128/jb.01319-10.

Jabłońska-Trypuć, A., Makuła, M., Włodarczyk-Makuła, M., Wołejko, E., Wydro, U., Serra-Majem, L. and Wiater, J. (2022). Inanimate Surfaces as a Source of Hospital Infections Caused by Fungi, Bacteria and Viruses with Particular Emphasis on SARS-CoV-2. *International Journal of Environmental Research and Public Health*, [online] 19(13), p.8121. doi:10.3390/ijerph19138121.

Jin, L., Li, W., Zhu, X., Zhao, H., Lu, Y., Zhang, C. and Lu, Z. (2019). Surfactin effectively inhibits Staphylococcus aureus adhesion and biofilm formation on surfaces. *Applied Microbiology and Biotechnology*, 103(11), pp.4565–4574. doi:10.1007/s00253-019-09808-w.

Kaan, T., Homuth, G., Mäder, U., Bandow, J. and Schweder, T. (2002). Genome-wide transcriptional profiling of the bacillus subtilis cold-shock response. *Microbiology*, 148(11), pp.3441–3455. doi:10.1099/00221287-148-11-3441.

Kayambankadzanja, R.K., Lihaka, M., Barratt-Due, A., Kachingwe, M., Kumwenda, W., Lester, R., Bilima, S., Eriksen, J. and Baker, T. (2020). The use of antibiotics in the intensive care unit of a tertiary hospital in Malawi. *BMC Infectious Diseases*, 20(1). doi:10.1186/s12879-020-05505-6.

Klein, E.Y., Van Boeckel, T.P., Martinez, E.M., Pant, S., Gandra, S., Levin, S.A., Goossens, H. and Laxminarayan, R. (2018). Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proceedings of the National Academy of Sciences*, 115(15), pp.E3463-E3470. doi:10.1073/pnas.1717295115.

Kramer, A., Schwebke, I. and Kampf, G. (2006). How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infectious Diseases*, [online] 6(1). doi:10.1186/1471-2334-6-130.

Méndez, M.B., Orsaria, L.M., Philippe, V., Pedrido, M.E. and Grau, R.R. (2004). Novel roles of the master transcription factors Spo0A and $\sigma^B$for survival and sporulation of*bacillus subtilis*at low growth temperature. *Journal of Bacteriology*, 186(4), pp.989–1000. doi:10.1128/jb.186.4.989-1000.2004.

Mhuireach, G., Dietza, L., Griffithsa, W., Finn Horvea, P., Laguerreb, A., Northcuttc, D., Vandegriftd, R., Gallb, E. and Van Den Wymelenberg, K. (2021). Differing effects of four building materials on viable bacterial communities and VOCs. *Developments in the Built Environment*, [online] 7(7), p.100055. doi:10.1016/j.dibe.2021.100055.

Neale, E.K. and Chapman, G.B. (1970). Effect of low temperature on the growth and fine structure of bacillus subtilis1. *Journal of Bacteriology*, 104(1), pp.518–528. doi:10.1128/jb.104.1.518-528.1970.

Nowroth, V., Marquart, L. and Jendrossek, D. (2016). Low temperature-induced viable but not culturable state of Ralstonia eutropha and its relationship to accumulated polyhydroxybutyrate. *FEMS microbiology letters*, [online] 363(23), p.fnw249. doi:10.1093/femsle/fnw249.

O'Neill, J. (2014). *Home | AMR Review*. [online] Amr-review.org. Available at: https://amr-review.org/ [Accessed 15 Sept. 2026].

Otter, J.A., Yezli, S. and French, G.L. (2011). The Role Played by Contaminated Surfaces in the Transmission of Nosocomial Pathogens. *Infection Control & Hospital Epidemiology*, [online] 32(07), pp.687–699. doi:10.1086/660363.

Petersen, E., Shui Shan Lee, Blumberg, L. and Levison, M.E. (2023). Antimicrobial resistance – a global problem in need of global solutions. *IJID Regions*, [online] 9(9), pp.102–103. doi:10.1016/j.ijregi.2023.10.005.

Piewngam, P., Khongthong, S., Roekngam, N., Theapparat, Y., Sunpaweravong, S., Faroongsarng, D. and Otto, M. (2023). Probiotic for pathogen-specific Staphylococcus aureus decolonisation in Thailand: a phase 2, double-blind, randomised, placebo-controlled trial. *The Lancet. Microbe*, [online] 4(2), pp.e75–e83. doi:10.1016/S2666-5247(22)00322-6.

Piewngam, P., Zheng, Y., Nguyen, T.H., Dickey, S.W., Joo, H.-S., Villaruz, A.E., Glose, K.A., Fisher, E.L., Hunt, R.L., Li, B., Chiou, J., Pharkjaksu, S., Khongthong, S., Cheung, G.Y.C., Kiratisin, P. and Otto, M. (2018). Pathogen elimination by probiotic Bacillus via signalling interference. *Nature*, 562(7728), pp.532–537. doi:10.1038/s41586-018-0616-y.

Sebiomo, A. (2020). Determination of exchangeable cations and residual concentration of herbicide treated soils and analysis of the in-vitro biodegradation of the herbicides. *Natural and Applied Sciences Journal*, 3(2), pp.49–63. doi:10.38061/idunas.759008.

Shimizu, M., Mihara, T., Ohara, J., Inoue, K., Kinoshita, M. and Sawa, T. (2022). Relationship between mortality and molecular epidemiology of methicillin-resistant Staphylococcus aureus bacteremia. *PLOS ONE*, 17(7), p.e0271115. doi:10.1371/journal.pone.0271115.

Silver, L.L. (2011). Challenges of antibacterial discovery. *Clinical microbiology reviews*, 24(1), pp.71–109. doi:10.1128/CMR.00030-10.

Tecon, R. and Or, D. (2016). Bacterial flagellar motility on hydrated rough surfaces controlled by aqueous film thickness and connectedness. *Scientific Reports*, [online] 6(1). doi:10.1038/srep19409.

The Lancet (2024). Antimicrobial resistance: a global health emergency. *The Lancet Respiratory Medicine*, [online] 12(11), pp.837–837. doi:10.1016/s2213-2600(24)00331-x.

Uju Marie-Esther, D. (2014). ISOLATION, CHARACTERIZATION AND BIODEGRADATION ASSAY OF GLYPHOSATE UTILIZING BACTERIA FROM EXPOSED RICE FARM . *International Journal of Scientific & Engineering Research*, 5(2), pp.1672–1700.

UKRI (2021). *GtR*. [online] Ukri.org. Available at: https://gtr.ukri.org/projects?ref=AH%2FR001987%2F1 [Accessed 16 Sept. 2026].

Weber, M.H., Klein, W., Müller, L., Niess, U.M. and Marahiel, M.A. (2001). Role of the bacillus subtilis fatty acid desaturase in membrane adaptation during cold shock. *Molecular microbiology*, [online] 39(5), pp.1321–9. doi:10.1111/j.1365-2958.2001.02322.x.

Weber, M.H.W. and Marahiel, M.A. (2002). Coping with the cold: The cold shock response in the gram-positive soil bacterium bacillus subtilis. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, [online] 357(1423), pp.895–907. doi:10.1098/rstb.2002.1078.

Wei, H., Scott, W., Hoenerloh, A., Leyton Dominguez, A., Nair, S. and Beckett, R. (2024). *Probiotic design interventions for the indoor microbiome community paper*.

WHO (2025). *Global COVID-19 Overview | WHO COVID-19 dashboard*. [online] datadot. Available at: https://data.who.int/dashboards/covid19/summary.

Wiegeshoff, F., Beckering, C.L., Debarbouille, M. and Marahiel, M.A. (2006). Sigma L is important for cold shock adaptation of *bacillus subtilis*. *Journal of Bacteriology*, 188(8), pp.3130–3133. doi:10.1128/jb.188.8.3130-3133.2006.

Wißmann, J.E., Kirchhoff, L., Brüggemann, Y., Todt, D., Steinmann, J. and Steinmann, E. (2021). Persistence of Pathogens on Inanimate Surfaces: A Narrative Review. *Microorganisms*, 9(2), p.343. doi:10.3390/microorganisms9020343.

Wollina, U. (2017). Microbiome in atopic dermatitis. *Clinical, Cosmetic and Investigational Dermatology*, Volume 10, pp.51–56. doi:10.2147/ccid.s130013.

World Bank (2025). *GDP (current US$)*. [online] The World Bank. Available at: https://data.worldbank.org/indicator/NY.GDP.MKTP.CD.

World Health Organization (2014). Antimicrobial resistance: global report on surveillance. *Who.int*. [online] doi:9789241564748.

World Health Organization (2023). *Antimicrobial resistance*. [online] World Health Organization. Available at: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.

World Health Organization (2024). *Global research agenda for antimicrobial resistance in human health*. World Health Organization.