DELIVERABLE D2.6: SUMMARY OF MAIN RESULTS ACHIEVED BY EACH CONTRACTOR AND CONCLUSIONS FROM PHASE 2

The main result for this second phase of the ANTISUPERBUGS PCP has been a ready-to-test working prototype, the BugWatcher ICT solution, to improve the quality of care-processes in hospital and the reduction of costs and collateral effects caused by Multi-Drug Resistant Organisms. This digital solution is composed of two main parts: first, two screening devices that allow the detection of the three target bacteria: paper strips for detection in humans and in the hospital fomites, and an air monitoring device to detect the bacteria in air. Secondly, it has an ICT Platform composed by a mobile application and a website, both addressed to healthcare workers, which will be in continuous communication with the hospital's HIS/LIS/EHR.
Regarding the different results achieved by our BugWatcher Consortium, we have divided them taking into account the different solution components.

Air monitoring screening device:
We have developed the airborne monitoring system concept outlined in Phase 1 into a fully functioning prototype in readiness for field test deployment. In addition, we have developed a prototype cartridge system which is required for the LM365 device to operate and which needs to be periodically exchanged. The cartridge system is required to supply the main sampling unit with freeze-dried reagents, primers and other chemistries required to facilitate the multiplexed NA (Nucleic Amplification) processes. As part of the design, the cartridge contains a microfluidic control system to facilitate the process

The LM365 device can reliably detect airborne pathogens including *Clostridium difficile*, *Klebsiella pneumoniae*, and methicillin-resistant *S. aureus* (MRSA). In addition, we have carried out some initial testing on viral pathogens, and completed testing with bacteriophages MS2 (E.coli host) and phi6 (Pseudomonas host) and the system design can also accommodate detection of additional pathogens in the future.

We have carried out a preliminary field deployment in Beaumont Hospital Dublin and the system performed as planned. We have also carried out internal and external laboratory testing to determine device efficacy and performance. For our phase 2 characterization of the device performance, Staphylococcus aureus (S. aureus) was chosen as a sample bacterium due to its prevalence in the hospital environment and frequent detection in air samples.

We have investigated the False Positive Rate for our assays and the True Negative Rate [Specificity] of our assays and we used a panel of interferences for mixed culture testing, including MSSA, E. coli, proteins and other matter collected from room air sampling. Our testing so far has not yet yielded any false positives or false negatives with the LAMP assays

Due to a number of significant innovations in the sampling and detection methods, we estimate the detection time to be < 90 min for a 60 m^2 room.

Lateral flow strips screening device:

We have identified three different targets to be detected using lateral flow strips (LFs), present in *Clostridium difficile*, methicillin-resistant *S. aureus* (MRSA) and *Klebsiella spp*. Several commercially available bioreceptors for these targets have been evaluated, finally obtaining a pair for each superbug which have been successfully integrated on the LFs. The current LFs prototypes exhibit the current detection limits: 14, 20 and 5 ng/mL for *Clostridium difficile*, MRSA and *Klebsiella spp*, respectively. Subsequently, as a part of the lab validation, the detection of the three bacteria was proved under BSL2 laboratory conditions using serial dilutions of reference strains from CECT (Spanish acronym of Spanish Type Culture Collection) type cultures.

Our LFs include magnetic beads (MBs) as colorimetric tags due the possibility to use this material as an element for removing the target from the sample, avoiding matrix effects and interferences. In addition, MBs can be used to pre-concentrate the sample before its addition to the LFs, helping to achieve lower detection limits. Sample collection can be performed by using buccal or rectal swabs in the case of patients, and surface swabs for fomites. As mentioned, the use of MBs will significantly reduce how the matrix medium may affect the performance of the assay with the LFs.

BugWatcher software (ICT component):

During this Phase 2, we have first designed and developed all the mobile app and website platform mock-up screens, considering the software architecture defined in Phase 1 and taking into account all the technological requirements from the Challenge Brief. After performing this "fine-tuning" and obtaining the final validated design of the components of the ICT Platform, we proceeded with the development of both mobile app and website platform.

The website platform is in charge of receiving all the alerts gathered by the screening devices, together with the adequate identifications (patient/fomite/room ID where the infection was detected, timestamp of the detection, type of contamination, source of information…). This information is sent and stored in our BugWatcher independent SQL-based database and can be sent to HIS/LIS/EHR, thanks to our MIRTH middleware connection. In the case of our working prototype, we have created a "fake HIS" to test this functionality and prior to Phase 3 we plan to conduct an iterative process of individual meetings to study, together with the pilot site their digital structure and architecture and adapt the integration with their system.

In the case of the mobile application, it is also addressed to healthcare workers and is in charge of gathering the results of the paper strips' tests performed in humans and in the hospital's fomites. HCW are able to register an infection (by taking a picture of the lateral flow test together with answering a short questionnaire with the results seen by the HCW by naked-eye) of a patient already registered in a specific room -by scanning his/her hospital wristband or by inserting manually all the identification data-, or the group of fomites inside a hospital location (room, hallway, waiting room, elevators zone…; wherever the sample was taken).

The involved actors in our BugWatcher solution, that will have access to both website platform and mobile application are (in order of hierarchy):

- **System Administrator**: main system developer. This user will own all of BugWatcher's permissions, including the creation or deletion of any type of users and the consultation or editing of their respective data. The administrators perform a complete management of the system at a technical level, in terms of IT and provide technical support to users if it is needed.
- **Chief/General Coordinator:** supervisor of the complete management program for each hospital. They have permissions to create and delete BugWatcher's users, such as doctors/nurses/microbiologists/maintenance staff on-demand. They are in charge of supervising the complete management of the field testing at a functional level in their own hospital center.
- **Doctor/Nurse**: they are able to log the detection of infections. They can log an infection in a room or in patients through the analysis of paper strips. They can do it manually via website or by the app selection or automatically scanning the patients' wristband.
- **Microbiologist**: They will be able to log information at molecular level on pathogens as well as validating infections and determining their virulence.
- **Maintenance staff**: The maintenance staff has access to the website platform but will only have permissions to see specific features of it. Mainly, they will receive a notification in case it is necessary to perform maintenance on the air monitoring systems. The platform will send a notification to the maintenance users in order to guarantee the correct function of the system.

Main conclusions of phase 2:

First and most important of all, we have obtained BugWatcher's ready-to-test working prototype, which includes both the ICT platform and the two screening devices. All the device integrations are also achieved and, like stated in other reports and in the above section, prior to Phase 3 we will organize individual meetings with each of the pilot sites in order to manage the integration with their HIS/LIS/EHR.

At the air monitoring device level, we have constructed our prototype device and undertaken internal and external lab efficacy testing and have been satisfied with the performance. We have also carried out a preliminary field deployment in Beaumont Hospital Dublin and the system performed as planned. During the course of Phase 2 we have continued to develop and refine the prototype ahead of field trials unit including preparing the device for compliance certification.

At the LFs level, we have confirmed the detection of the three bacteria in BSL2 environment, although in the case of *Klebsiella spp* an improvement may be needed, which could include subcontracting a company to develop specific bioreceptors for *Klebsiella pneumoniae* for LPS (lipopolysaccharide), CPS (capsular polysaccharide with several serotypes), O (O-specific polysaccharides or O-side chain) antigens or other antigenic regions. Additionally, we have reconsidered the use of the vacuum cleaner for collecting fomites samples. Instead, in order to avoid aerosol generation, to be more easily implemented in the hospital workflow and for a better collection of the sample, we recommend using a surface swab. Therefore, the LFs workflow will be the same por patients and fomites, greatly simplifying the actions for the user.

Finally, at ICT level we validated and confirmed the role of all users, we designed and developed the alerts system, together with the website platform and mobile application included in BugWatcher.

In summary, all the Consortium members are fully aligned and ready to perform the field testing of our solution.