hygiene

MDPI

Review

Down the Drain: A Systematic Review of Molecular Biology Evidence Linking Sinks with Bacterial Healthcare-Associated Infections in Intensive Care Units

Morgane Choquet [1,2] and Catherine Mullié [1,3,*]

[1] Laboratoire AGIR-UR UPJV 4294, UFR de Pharmacie, Université de Picardie Jules Verne, 80037 Amiens, France; choquet.morgane@chu-amiens.fr
[2] Laboratoire de Bactériologie, Centre Hospitalier Amiens-Picardie, 80025 Amiens, France
[3] Laboratoire Hygiène, Risque Biologique et Environnement, Centre Hospitalier Universitaire Amiens-Picardie, 80025 Amiens, France
* Correspondence: catherine.mullie@u-picardie.fr

Abstract: This systematic review aimed to sum up the evidence gathered by molecular biology methods on the transmission of bacterial clones from sinks/sink drains environmental sources to intensive care unit (ICU) patients. Forty-five reports meeting inclusion/exclusion criteria were identified. Most were retrospective cohort studies on Gram negative multidrug resistant bacteria, with *P. aeruginosa* and *S. marcescens* being the most frequent species (26.7% and 17.8% of the studies, respectively). The reports using pulse field gel electrophoresis were the most numerous (44.4%) and found a common clone between clinical and sink/sink drains isolates in 80% of the cases. Over the last 5 years, the use of whole genome sequencing became more frequent and linked sink/sink drains isolates to clinical ones in 50% of the cases. Precise positivity timelines mostly pointed towards a patient-to-sink/sink drain transmission while only 8 reports provided back up for the sink/sink drain-to-patient. To better appraise the role of sinks/sink drains as a reservoir for nosocomial acquisition of bacteria in ICU, future reports should strive to give a precise timeline for the retrieval of isolates as well as the cut-off criteria used to assign isolates to a given clone (information lacking in 66.7% and 42.2% of the studies, respectively).

Keywords: sink; sink drain; bacterial dissemination; hospital acquired infection; outbreak; intensive care unit; molecular typing; pulse field gel electrophoresis; rep-PCR; whole genome sequencing

Citation: Choquet, M.; Mullié, C. Down the Drain: A Systematic Review of Molecular Biology Evidence Linking Sinks with Bacterial Healthcare-Associated Infections in Intensive Care Units. *Hygiene* **2022**, *2*, 94–108. https://doi.org/10.3390/hygiene2020008

Academic Editor: Günter Kampf

Received: 9 May 2022
Accepted: 4 June 2022
Published: 8 June 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Patients hospitalized in intensive care units (ICUs) are acknowledged as having a higher risk of healthcare-associated infections (HAIs) than other patients [1,2]. This higher risk has been linked with multiple risk factors such as length of stay, inadequate and/or long-term antibiotic administration, assisted-ventilation, or catheters [3,4]. Moreover, those HAIs are often caused by multidrug resistant (MDR) bacteria, leading to struggles in efficiently tackling those infections in already critically ill patients and even sometimes to therapeutic dead-ends [1,5]. It is therefore of utmost importance to better understand how bacterial HAIs are acquired by ICU patients to try and prevent their occurrence. Two main routes of contamination are classically described: (i) autoinfection where patients are contaminated by bacteria they themselves brought in the healthcare facility (in their intestinal or nasal microbiota, for example) and (ii) cross-contamination where bacteria are acquired directly or indirectly from other patients, healthcare workers, the environment, and/or medical devices [6]. Regarding cross-contaminations, recommendations and guidelines have been published worldwide to mitigate this known route of transmission, especially through hand hygiene good practices [6,7]. However, direct or indirect cross-contamination via the environment (e.g., through water, air, or high-touch surfaces)

is not so readily recognized as an important transmission route [8]. Amongst possible environmental sources of contamination, sinks and more specifically sink drains have been pointed out but their role is still debated [9,10]. One of the major hurdles in assessing the importance of this contamination source is the ability to assign bacteria isolated from patients and the presumed environmental source to a same bacterial clone and, taking into account the chronology, establish a cause-effect link. Phenotypic characters, including antibiotic resistance profiles, are not discriminatory enough to ascertain that isolates belong to a same clone, as previously demonstrated [11]. Molecular biology techniques are more suited to this purpose. The most frequently used mainly rely on (i) comparison of DNA electrophoretic profiles obtained from either native/restriction enzyme-treated DNA (e.g., Restriction Endonuclease Analysis, Pulsed-Field Gel Electrophoresis), or PCR amplified DNA (e.g., Random Amplified Polymorphic DNA, Repetitive element palindromic-PCR) and (ii) partial (MultiLocus Sequence Typing) or complete (Whole Genome Sequencing) genome sequencing of the isolates followed by comparison of sequences against online databases and/or between isolates. This review therefore analyzed the available data on HAIs (especially outbreaks) in ICUs linking an environmental "sink" source to infected patients through molecular biology methods to help clarify the importance of this contamination source.

2. Materials and Methods

This systematic review was performed according to the PRISMA guidelines [12] using Pubmed, EMBASE, and LILACS databases. As this work did not perform a systematic assessment of human intervention clinical trials or a meta-analysis per se, it was not registered online in a repository. The search strategy was ("sink" [All fields]) AND ("intensive care unit" [All fields]) AND ("outbreak" [All fields]). Original studies were eligible if published between 1 January 1990 and 30 November 2021. The search was last run against the databases on 29 December 2021. The other inclusion criteria were: any type of study design (randomized clinical trials, cohort studies, case-control studies, observational studies, outbreak investigations) and use of a molecular biology technique to attribute a bacterial isolate to a given clone. Studies with an English abstract were eligible when published in English, French, or Spanish. Publications were not eligible if they consisted in reviews, commentaries and other opinion papers, reported on outbreaks of viral, fungal, or parasitic pathogens, were held in healthcare settings other than ICUs or did not include a molecular biology technique for the identification of bacterial isolates at the clonal level. The primary outcome is the identification of the same clone in environmental sink samples (sink, sink drain, sink trap, sink bowl, sink tap/faucet) and in patient samples. The secondary outcome is the chronology between retrieval of isolates from environmental and clinical samples.

After retrieval of results from database searches, each reviewer independently screened the papers on the basis of the title and abstract to see if they could answer the purpose of this review. Discrepancies in the list of papers to be included in the full-text article evaluation were then discussed and a common final decision was taken. Similarly, the list of selected studies was also jointly reappraised after reading the full texts of those full-texts.

The following information were sought and registered whenever available: type of study, type of ICU (neonatal/pediatric intensive care unit (NICU/PICU) or adult intensive care unit), species of bacteria involved, molecular biology technique used for the typing of isolates and decision criteria to attribute strains to a clone, sampling chronology, number of clinical samples included, number of environmental samples included, length of the outbreak, and mitigation interventions.

3. Results

3.1. Identification and Selection of Studies

Once the selection process was completed, 45 full-text entries were included in the analysis (Figure 1). An increase in the number of papers published on this subject is observed, with the last decade being the most prolific (Figure 2).

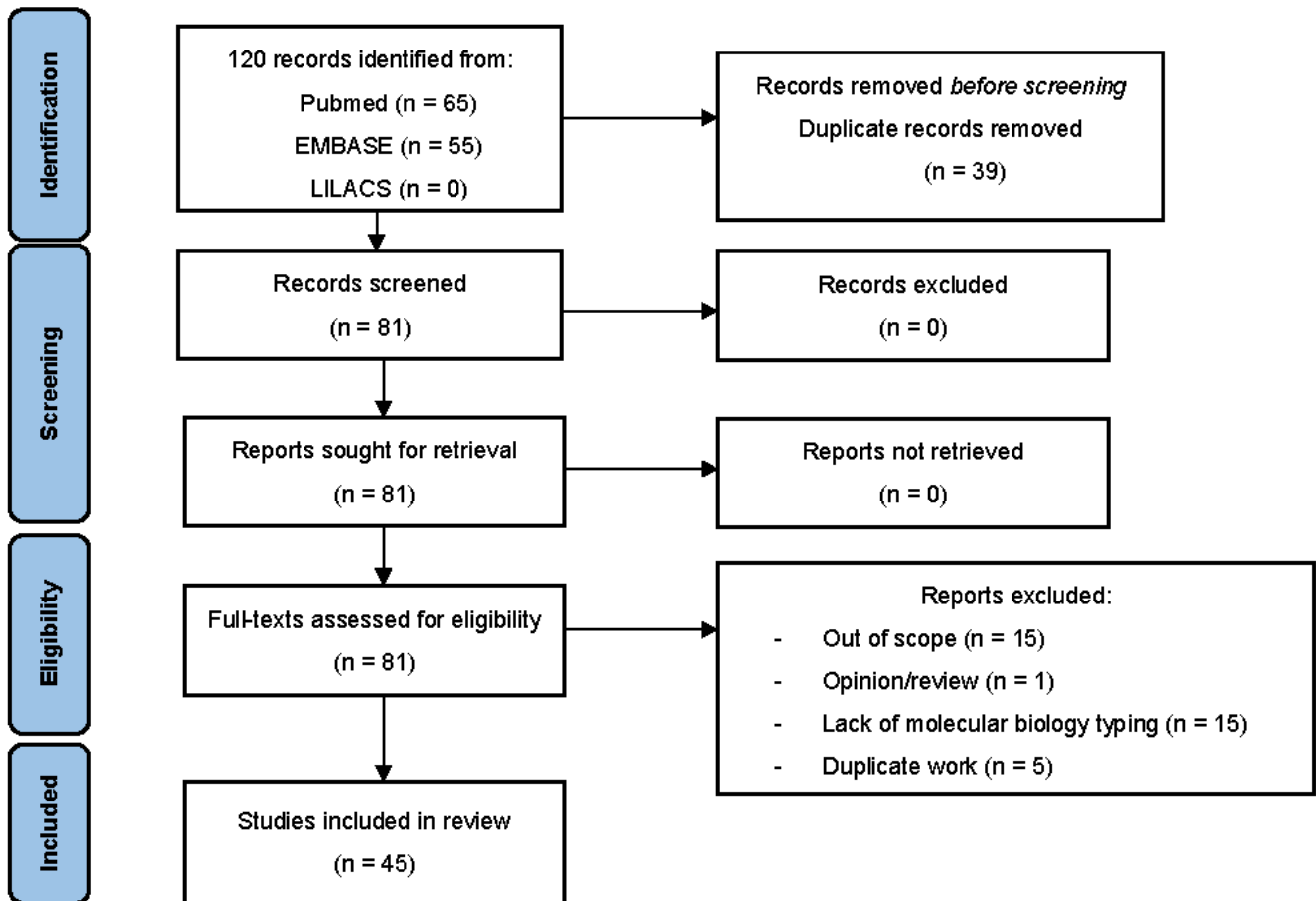

Figure 1. Molecular biology evidence linking sinks with bacterial nosocomial outbreaks in intensive care units; Systematic review-PRISMA flow chart.

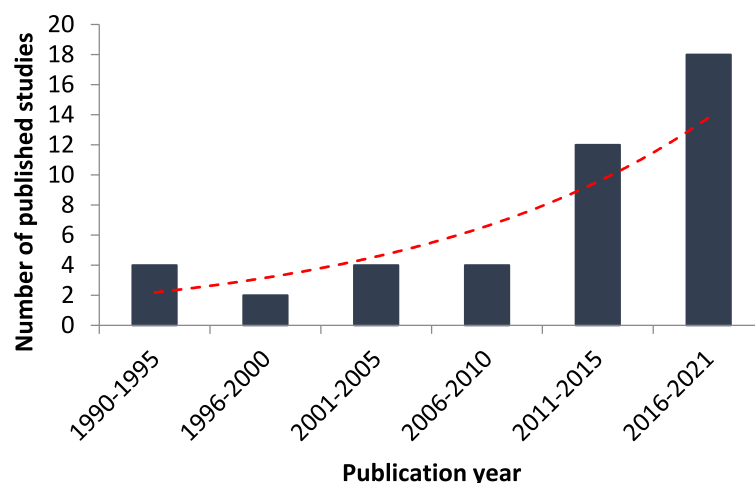

Figure 2. Number of published papers reporting molecular biology identification of bacterial isolates retrieved from clinical and sink samples in ICUs. The dotted red line represents the computed trend curve of the number of publications over the 1990–2021 period.

Most of these studies were observational, retrospective, and monocentric, being held after an outbreak (35/45, 77.8%) (Table 1). Only a few were fully prospective (5/45, 11.1%) [11,13–16] and a single retrospective cohort study was bicentric (2.2%) [17]. One

report did not include sufficient information to categorize the study design [18] while three others mixed retrospective with prospective acquisition of data [9,19,20].

3.2. Main Characteristics of the Selected Studies

These characteristics are summarized in Table 1. Overall, 16 studies (35.6%) were held in NICU/PICU. Their durations ranged from 2 weeks [17] up to 11 years [21].

Table 1. Main characteristics of the studies included in the review.

Reference	Setting	Type of Study	Duration (Outbreak and/ or Study)	Molecular Biology Technique(s)	Species/Group of Species Investigated
McGeer et al. [11]	NICU [1]	Prospective	8 months	REA [1]	*Serratia marcescens*
Orr et al. [22]	ICU [1]	Retrospective	19 days	Pyrolysis Mass Spectrometry	*Stenotrophomonas maltophilia*
Döring et al. [13]	ICU [1]	Prospective	3 months	Exotoxin A DNA probe	*Pseudomonas aeruginosa*
Kerr at al. [23]	ICU [1]	Retrospective	3 months	RAPD [1]	*Pseudomonas aeruginosa*
Verweij et al. [24]	NICU [1]	Retrospective	3 months	RAPD [1]	*Stenotrophomonas maltophilia*
Su et al. [25]	PICU [1]	Retrospective	Not provided	Infrequent restriction site PCR [1] PFGE [1]	*Klebsiella pneumoniae*
Hoque et al. [26]	NICU [1]	Retrospective	21 months	PFGE [1]	*Elizabethkingia meningoseptica*
van Dijk et al. [27]	NICU [1]	Retrospective	11 months	AFLP [1] Rep-PCR [1]	*Enterobacter cloacae*
Wagenlehner et al. [18]	ICU [1]	Retrospective	19 months	PFGE [1]	*Enterobacter* spp.
Milisavljevic et al. [17]	NICUs [1]	Retrospective	2 weeks (NICU [1] A) 1 month (NICU [1] B)	PFGE [1]	*Serratia marcescens*
Kac et al. [28]	ICU [1]	Retrospective	16 months	RAPD [1]	ESBL [1]-producing enterobacteria
Maragakis et al. [29]	NICU [1]	Retrospective	5 months	PFGE [1]	*Serratia marcescens*
Hota et al. [30]	ICU [1]	Retrospective	28 months	PFGE [1]	*Pseudomonas aeruginosa*
LaForgia et al. [31]	ICU [1]	Retrospective	10 months	REA [1]	*Acinetobacter baumannii*
Longtin et al. [21]	PICU [1]	Retrospective	30 months	PFGE [1]	*Pseudomonas aeruginosa*
Lucero et al. [32]	NICU [1]	Retrospective	12 months	Rep-PCR [1]	*Burkholderia cepacia* complex
Guleri et al. [33]	ICU [1]	Retrospective	5 months	Variable Number Tandem Repeat	*Pseudomonas aeruginosa*
Diederen et al. [34]	ICU [1]	Retrospective	61 months	Unspecified molecular biology typing	*Pseudomonas aeruginosa*
Maltezou et al. [35]	NICU [1]	Retrospective	34 months	PFGE [1]	*Serratia marcescens*
Lowe et al. [36]	ICU [1]	Retrospective	54 months	PFGE [1]	*Klebsiella oxytoca*
Starlander et al. [37]	ICU [1]	Retrospective	7 months	PFGE [1]	*Klebsiella pneumoniae*
Kotsanas et al. [38]	ICU [1]	Retrospective	33 months	PFGE [1]	CPE [1]
Landelle et al. [39]	ICU [1]	Restrospective	17 months	PFGE [1]	*Acinetobacter baumannii*
Vergara-Lopez et al. [40]	ICU [1]	Retrospective	33 months	PFGE [1]	*Klebsiella oxytoca*
Wendel et al. [19]	ICU [1]	Retro- & Prospective	11 years	MLST [1]	*Pseudomonas aeruginosa*
Davis et al. [41]	PICU [1]	Retrospective	4 months	Rep-PCR [1] WGS [1]	*Pseudomonas aeruginosa*
Umezawa et al. [42]	ICU [1]	Retrospective	1 month	Rep-PCR [1] MLST [1]	*Acinetobacter baumannii*
Zhou et al. [14]	ICU [1]	Prospective	8 months	PFGE [1]	*Pseudomonas aeruginosa*
Salm et al. [43]	ICU [1]	Retrospective	28 months	Rep-PCR	*Pseudomonas aeruginosa*
De Geyter et al. [44]	ICU [1]	Retrospective	8 months	PFGE [1]	CPE [1]

Table 1. *Cont.*

Reference	Setting	Type of Study	Duration (Outbreak and/or Study)	Molecular Biology Technique(s)	Species/Group of Species Investigated
Herruzo et al. [45]	NICU [1]	Retrospective	5 months	Rep-PCR [1]	*Klebsiella oxytoca*
Cantero et al. [46]	ICU [1]	Retrospective	6 weeks	PFGE [1]	*Chryseobacterium indologenes*
Regev-Yochay et al. [47]	ICU [1]	Retrospective	17 months	PFGE [1]	*Serratia marcescens*
Martischang et al. [48]	ICU [1]	Retrospective	2 consecutive summers	WGS [1]	*Serratia marcescens*
Eveillard et al. [9]	ICU [1]	Retro- & Prospective	5 years	PFGE [1]	*Klebsiella pneumoniae*
Avaness et al. [49]	ICU [1]	Retrospective	3 months	Plasmid analysis	CPE [1]
Jung et al. [50]	ICU [1]	Retrospective	6 months	PFGE [1]	CPE [1]
Yeo et al. [51]	NICU [1]	Retrospective	4 months	Rep-PCR [1] WGS [1]	*Serratia marcescens*
de Man et al. [52]	ICU [1]	Retrospective	10 months	WGS [1]	CPE [1]
Qiao et al. [15]	ICU [1] & NICU [1]	Prospective	6 months	WGS [1]	*Klebsiella oxytoca* & *Klebsiella pneumoniae*
Lemarié et al. [16]	ICU [1]	Prospective	12 months	PFGE [1]	CPE [1]
Gideskog et al. [53]	ICU [1]	Retrospective	1 month	WGS [1]	*Stenotrophomonas maltophilia*
Blanc et al. [20]	NICU [1]	Retro- & Prospective	4 months	WGS [1]	*Pseudomonas aeruginosa*
Catho et al. [54]	ICU [1]	Retrospective	31 months	WGS [1]	*Pseudomonas aeruginosa*
Bourdin et al. [55]	NICU [1]	Retrospective	Not provided	HiSST [1]	*Serratia marcescens*

[1] Abbreviations used in this table: AFLP = Amplified Fragment Length Polymorphism; CPE = Carbapenemase-Producing Enterobacteria; ESBL = Extended Spectrum Beta-Lactamase; HiSST = High-throughput Short Sequence Typing; ICU = Intensive Care Unit (adults); MLST = MultiLocus Sequence Typing; NICU = Neonatal Intensive Care Unit; PCR = Polymerase Chain Reaction; PFGE = Pulsed-Field Gel Electrophoresis; PICU = Pediatric Intensive Care Unit; RAPD = Random Amplified Polymorphic DNA; REA = Restriction Endonuclease Analysis, Rep-PCR = Repetitive element palindromic-PCR; WGS = Whole genome sequencing ± in silico MultiLocus Sequence Typing.

3.3. Risk of Bias and Quality Assessment of the Selected Studies

No readily available tool for assessment of risk of bias and quality, be it a scale or a check list, was found that could match this systematic review purpose, which focused on molecular biology investigation of bacterial isolates. Therefore, a simple checklist including four criteria deemed essential for a valid use of molecular biology techniques and establishing a link between clinical and environmental (sink/sink drains) isolates was set up to assess the quality of studies included in this review (Table 2). The study design was also included to account for risk of bias inherent to each one of the study designs. An overall quality score was then calculated, the maximum score value being of 7 (Table 2). The median quality score calculated was of 3 and scores ranged between 1 and 6. The quality score was classified as low for 42.2% (19/45), fair for 46.7% (21/45), and good for 11.1% (5/45) of the studies, respectively (Table 2).

3.4. Bacterial Species Involved

Reports on enterobacteria were the more numerous, representing 57.8% (26/45) of the studies analyzed while the 21 remaining reports dealt with Gram negative non-fermentative bacteria (Table 3). No studies linking Gram positive bacteria outbreaks to sinks/sink drains were retrieved with the search strategy employed for this review. At the species level, *Pseudomonas aeruginosa* garnered the most reports (12) followed by *Serratia marcescens* (8) (Table 3).

Table 2. Quality assessment of the included studies regarding timeline and molecular biology technique reporting.

Reference	Study Design [1]	Precise Clinical and Environmental Positivity Timeline [2]	Molecular Biology Technique Cut-Off Threshold Provided [2]	Molecular Biology Analysis of All Isolates Reported [2]	External Control Strain(s) [2]	Overall Score [3]
McGeer et al. [11]	Prospective	1	0	0	0	4
Orr et al. [22]	Retrospective cohort	0	1	0	1	3
Döring et al. [13]	Prospective	1	0	0	0	4
Kerr at al. [23]	Retrospective cohort	0	1	1	1	4
Verweij et al. [24]	Retrospective cohort	1	1	1	1	5
Su et al. [25]	Retrospective cohort	0	1	1	1	4
Hoque et al. [26]	Retrospective cohort	1	0	0	0	2
van Dijk et al. [27]	Retrospective cohort	0	0	0	1	2
Wagenlehner et al. [18]	Retrospective cohort	0	1	0	1	3
Milisavljevic et al. [17]	Retrospective cohort	0	1	1	0	3
Kac et al. [28]	Retrospective cohort	0	1	1	0	3
Maragakis et al. [29]	Retrospective cohort	0	1	0	0	2
Hota et al. [30]	Retrospective cohort	0	0	0	0	1
LaForgia et al. [31]	Retrospective cohort	1	1	0	0	3
Longtin et al. [21]	Retrospective cohort	0	1	0	0	2
Lucero et al. [32]	Case-control	0	1	0	0	3
Guleri et al. [33]	Retrospective cohort	0	0	0	0	1
Diederen et al. [34]	Case-control	0	0	0	0	2
Maltezou et al. [35]	Case-control	0	1	1	0	4
Lowe et al. [36]	Retrospective cohort	0	0	1	0	2
Starlander et al. [37]	Retrospective cohort	0	0	0	0	1
Kotsanas et al. [38]	Retrospective cohort	0	1	0	0	2
Landelle et al. [39]	Retrospective cohort	1	0	0	0	2
Vergara-Lopez et al. [40]	Retrospective cohort	1	0	0	0	2
Wendel et al. [19]	Retrospective cohort [4]	1	1	0	0	3
Davis et al. [41]	Retrospective cohort	1	1	0	0	3
Umezawa et al. [42]	Retrospective cohort	0	1	1	0	3
Zhou et al. [14]	Prospective	1	1	1	0	6
Salm et al. [43]	Case-control	0	1	1	0	4
De Geyter et al. [44]	Retrospective cohort	0	0	0	0	1
Herruzo et al. [45]	Retrospective cohort	1	0	1	0	3
Cantero et al. [46]	Retrospective cohort	0	0	0	0	1
Regev-Yochay et al. [47]	Retrospective cohort	0	0	0	0	1
Martischang et al. [48]	Retrospective cohort	0	0	0	0	1
Eveillard et al. [9]	Retrospective cohort [4]	0	1	0	0	2
Avaness et al. [49]	Retrospective cohort	0	0	0	0	1
Jung et al. [50]	Case-Control	1	0	0	0	3
Yeo et al. [51]	Retrospective cohort	1	1	1	1	5
de Man et al. [52]	Retrospective cohort	0	0	0	1	2
Qiao et al. [15]	Prospective	1	1	1	0	6
Lemarié et al. [16]	Prospective	0	1	1	0	5
Gideskog et al. [53]	Retrospective cohort	0	1	1	0	3
Blanc et al. [20]	Retrospective cohort [4]	0	1	1	0	3
Catho et al. [54]	Retrospective cohort	1	1	0	1	4
Bourdin et al. [55]	Retrospective cohort	0	1	0	1	3
Overall reporting frequency		**33.3% (15/45)**	**57.8% (26/45)**	**35.6% (16/45)**	**22.2% (10/45)**	

[1] Scores for designs were 3 for prospective studies, 2 for case-control studies, and 1 for retrospective cohort studies. [2] For each item, a "yes" answer was scored 1 and a "no" answer scored 0. [3] The overall score was calculated by adding up the scores obtained for individual criteria, with a possible maximum value of 7. A study report was evaluated as of (i) good quality for an overall score ranging from 5 to 7, (ii) fair quality for overall scores of 3 or 4 and (iii) low quality for scores ranging from 0 to 2. [4] Prospective acquisition of data was also carried out in these studies.

Table 3. Bacterial species/groups found in HAIs with a possible sink origin.

Group/Species	Antibiotic Resistance Profile	Possible Link [1,2]	No Proven Link [2]
Non-fermentative Gram-negative bacteria			
Acinetobacter baumannii	MDR [3]	REA [3] [31]	-
		PFGE [3] [39]	-
	AM, CIP [3]	MLST [3] & Rep-PCR [3] [42]	-
Burkholderia cepacia complex	Not reported	Rep-PCR [32]	-
Chryseobacterium indologenes	Not reported	PFGE [46]	-
Elizabethkingia meningoseptica	MDR	PFGE [26]	-
Pseudomonas aeruginosa	Not reported	Exotoxine A DNA probe [13] PFGE [14]	-
		RAPD [3] [23]	
	Intrinsic resistance only	PFGE [21] Rep-PCR & WGS [3] [41]	WGS [20]
	GIM [3]-1 carbapenemase	MLST [19]	-
	MDR	PFGE [30] Rep-PCR [43]	Variable number tandem-repeat [33]
	VIM [3] carbapenemase	WGS [54]	Unspecified molecular typing method [34]
Stenotrophomonas maltophilia	Susceptible to TMP-SFX [3]	WGS [53]	WGS [53]
	Not reported	RAPD [24]	Pyrolysis mass spectrometry [22]
Enterobacteria	OXA [3]-48 carbapenemase	-	PFGE [16]
	VIM carbapenemase	-	WGS [52]
	Carbapenemase-producing	PFGE [50]	PFGE [38,44] Plasmid analysis [49]
	ESBL [3]	RAPD [28]	-
Citrobacter freundii	OXA-48 carbapenemase	PFGE [44]	-
Enterobacter spp.	Variable	PFGE [18]	-
Enterobacter cloacae	ESBL	-	Rep-PCR [27]
Klebsiella oxytoca	VIM carbapenemase	-	Rep-PCR [45]
Klebsiella pneumoniae	IMP [3]-8 carbapenemase	PFGE [40]	-
	Carbapenem-resistant	WGS [15]	-
	ESBL	PFGE [36]	-
	OXA-48 carbapenemase	PFGE [9]	-
	Carbepenem-resistant	WGS [15]	-
	ESBL	PFGE [37]	-
	Variable	PFGE [25]	
Serratia marcescens	Not provided	HiSST [55]	Rep-PCR and WGS [51]
	Intrinsic resistance only	PFGE [35]	PFGE [29]
	MDR	-	PFGE [17]
	Variable	REA [11]	WGS [48]
	OXA-48 carbapenemase	PFGE [47]	-

[1] A link was considered as possible when the same genotype/clone was isolated from at least a clinical sample and a sink sample. [2] The method used to assign isolates to a clone is specified for each reference in the column. [3] Abbreviations used in this table: AM = Amikacin, CIP = Ciprofloxacin, ESBL = Extended-Spectrum β-Lactamase, GIM = German Imipenemase, HiSST = High-throughput Short Sequence Typing, IMP = Imipenemase, MDR = Multidrug Resistant, MLST = MultiLocus Sequence Typing, OXA = Oxacillinase, PFGE = Pulsed-Field Gel Electrophoresis, RAPD = Random Amplified Polymorphic DNA, REA = Restriction Endonuclease Analysis, Rep-PCR = Repetitive element palindromic-PCR, TMP-SFX = Trimethoprim-Sulphamethoxazole, VIM = Verona Integron-Encoded Metallo-β-Lactamase, WGS = Whole genome sequencing ± in silico MultiLocus Sequence Typing.

3.5. Molecular Typing Methods Used for Attribution of Bacterial Isolates to Clones

3.5.1. Random Amplified Polymorphic DNA (RAPD)

Three (6.7%) studies reported results using this method [23,24,28]. It was used to discriminate strains belonging to *P. aeruginosa* [23] and *Stenotrophomonas maltophilia* species [24] while the last work focused on extended spectrum beta-lactamase (ESBL) producing en-

terobacteria and used RAPD to discriminate *Citrobacter freundii* isolates. The criteria to distinguish between clones were not similar in two of the studies: while one attributed isolates to a single clone when RAPD profiles showed a difference of less than three bands [28], the other stated that genotypes were characterized as identical (identical banding pattern), highly related (one mismatch in banding pattern) or unrelated (two or more mismatches) [24]. The third report did not specify their strategy to assign isolates to clones/genotypes [23].

3.5.2. Restriction Endonuclease Analysis (REA) and Pulse Field Gel Electrophoresis (PFGE)

REA and PFGE were used to assign bacterial isolates to clones in 22 out of the 45 studies analyzed (48.9%). REA was used in only two studies on *S. marcescens* and *Acinetobacter baumannii*, respectively [11,31]. No cut-off for discriminating between clusters/clones was provided in the first one [11] while the second proposed a similarity index of at least 90% to attribute two isolates to a same clone [31]. PFGE was used in 20 studies on various microorganisms (4 studies for *S. marcescens*; 3 for carbapenem-producing enterobacteria and *P. aeruginosa*; 2 for *Klebsiella pneumonia* and *K. oxytoca*; 1 for *Chryseobacterium indologenes*, *C. freundii*, *A. baumannii*, *Enterobacter* spp., *Elizabethkingia meningoseptica* and *Burkholderia cepacia* complex, respectively). However, the cut-off criteria employed for the assignment of an isolate to a clone could vary from one report to another. Nine papers directly or indirectly reporting cut-off criteria referred to the ones proposed by Tenover et al. [56] with strains classified as indistinguishable when restriction patterns show the same number of bands with the same apparent sizes, closely related when a maximum difference of three bands is registered, possibly related when a four to six band difference in patterns is witnessed and unrelated when seven or more band differences are found. Three reports either stated that isolates with identical profiles were part of a same clone [14], or used a Dice index superior to 90% [39], or stated that Tenover criteria were too stringent for a proper clonal assignment and used a biometric software instead without providing a cut-off value [30]. The remaining reports did not present cut-off values [26,32,36,40,44,46,47,50].

3.5.3. Repetitive Element Palindromic-PCR (Rep-PCR)

Seven occurrences (15.6%) of its use were found in the analyzed studies, 2 for *P. aeruginosa* typing [41,43] and one for each of the following species or complexes: *S. marcescens* [51], *K. oxytoca* [45], *A. baumannii* [42], *E. cloacae* [27] and *B. cepacia*/*B. cenocepacia* complex [32]. Profile similarity percentages of 94% [51] or at least 95% [42,43] were set as cut-offs to discriminate between clones. However, some of the reports only stated the name of the software used to delineate clones [45] and/or did not specify the values they used for cut-offs [27,32,41].

3.5.4. MultiLocus Sequence Typing (MLST)

Actual MLST (as opposed to in silico MLST following Whole Genome Sequencing, cf. 3.2.4.) was only reported twice for the attribution of *P. aeruginosa* [19] and *A. baumannii* [42] isolates to Sequence Types (STs) previously described in existing MLST schemes.

3.5.5. Whole Genome Sequencing (WGS) and In Silico MLST

Nine of the more recent papers report the use of WGS. *S. marcescens* [48,51,55] and *P. aeruginosa* [20,41,54] were each investigated in three studies while *S. maltophilia* [53], *K. oxytoca* and *K. pneumoniae* [15] as well as VIM-producing enterobacteria [52] were each the focus of one report. For this technique, the cut-off values to separate clones are expressed as the number of Single Nucleotide Polymorphisms (SNPs) present between genomes. This cut-off value can vary from one bacterial species to the other, depending on the estimated genome mutation rate for each species. For example, Qiao et al. [15] chose a cut-off value of 21 SNPs to define isolates belonging to a same clone of *K. pneumoniae* while Yeo et al. [51] validated an upper threshold of 6 SNPs for *S. marcescens* isolates to belong to a same clone.

Core genome-sequencing also allows for the in silico determination of ST when an MLST scheme exists, which has been performed in eightstudies out of nine studies.

3.5.6. Miscellaneous Techniques

Only one published occurrence was retrieved for several molecular typing techniques such as: exotoxine A DNA probe [13], pyrolysis mass spectrometry [22], Variable number tandem-repeat (VNTR) molecular typing [33], and plasmid analysis [49]. Finally, a new method named high-throughput short sequence typing (HiSST) and based on the sequence differences obtained from three highly variable loci was recently described for *S. marcescens* [55].

3.6. Link between Sink and Patients Isolates

As can be seen in Table 3, these different molecular typing techniques led to the conclusion of a possible link between environmental and clinical isolates in 31 (68.8%) out of the 45 studied papers. The study published by Gidesock et al. [53] reported that although one *S. maltophilia* clone infecting patients could not be recovered from sink samples, the other outbreak clone was isolated from a sink sample as well as one patient. This mixed result generated 16 (35.6%) occurrences out of the 45 studied papers in which the outbreak clone was not the same as the one isolated from a sink/sink drain sample. Among the most frequently used molecular biology techniques, the attribution of environmental and clinical isolates to the same clone was made in 80% (16/20) of the studies using PFGE while it was only made in 50% (5/10) of the studies using WGS.

It is worth noticing that the chronology of isolation from environmental and patient samples was not always clearly provided in the 31 studies with a clone found in common in both types of samples (Tables 2 and 3). Most studies were retrospective and thus implied that environmental sampling took place after the first isolates were cultured from clinical samples, without further precision. They were therefore indicative of a possible transmission of the bacterial clone from patients to sinks/sink drains but not conclusive for a transmission occurring the other way around. Only eight studies were prospective and/or provided precise sampling time-points and, based on the positive sampling chronology, advocated for a possible transmission from sinks/sink drains to patients [13–15,19,31,32,40,50]. Also, although not providing exact sampling times, Salm et al. [43] as well as Diederen et al. [34] conducted retrospective case-control studies and found that staying in a room with a colonized sink was independently linked with an elevated risk for colonization or infection by a *P. aeruginosa* outbreak strain.

4. Discussion

Protracted and discontinuous outbreaks of MDR bacteria in absence of a temporal overlap of clinical cases point to an environment-to-patient transmission rather than a person-to-person contamination. Environmental investigations are therefore often performed in such contexts. Sinks, sink drains and sink-associated surfaces are frequently sampled in such investigations due to the propensity of some known bacterial species such as *P. aeruginosa* and enterobacteria to persist in these wet environments [57–60]. Indeed, sink drains in hospitals have long been known to harbor an abundant microbiota comprising up to 10^3–10^5 CFU/mL Gram-negative rods, especially waterborne bacteria [61,62]. It is noteworthy that although meticillin-resistant *Staphylococcus aureus* (MRSA) or Vancomycin-Resistant Enterococci (VRE) outbreaks do occur in ICUs, none of the papers analyzed in this review related to Gram-positive bacteria. Indeed, very few papers deal with the contamination of sinks/sinks drains with Gram-positive bacteria as the main mode of transmission of these pathogens is thought to be a person-to-person transmission [63,64]. However, environment-driven transmission has also already been described as possible for both MRSA and VRE [65,66].

Going back to sink drains bacterial contamination, getting rid of a bacterial colonization in sink drains, especially if the colonizing species is prone to develop biofilms, has

been described as extremely difficult [16,36,47,60,67]. Nevertheless, the debate on the role of such reservoirs in the dissemination of MDR bacteria in healthcare settings and their implication in HAIs is still on-going, especially in patients with multiple risk factors such as those housed in ICUs. The aim of this work was to review the evidence provided by molecular biology techniques linking bacterial isolates from sinks/sink drains and ICU patient isolates. An acknowledged weakness of the chosen search strategy is the inclusion of "outbreak" as a keyword. This would indeed skew the search results towards retrospective studies carried out after an outbreak. However, as stated above, sampling the environment in search of MDR bacteria is mostly done in such cases. In addition, we were still able to retrieve a few prospective studies on the subject [11,13–16] through this strategy as well as a couple of studies which started retrospectively but were prolonged prospectively [9,19,20].

In addition, the information harvested through this investigation enabled us to point out several facts including the increase in recent reports over the subject (Figure 2) and the changes in molecular typing methods occurring over time. PFGE was referred to as the gold standard for clone discrimination from 2000 until the second part of the 2010s. After a brief incursion into partial genome sequencing through the use of MLST, WGS is currently taking the front place. This evolution is driven by the now lower cost of sequencing provided by next generation sequencing techniques and the need for more discriminatory methods. Apart from MLST, which relies on libraries/schemes available online to attribute an isolate to a previously described Sequence Type (ST) or a new one, most of these techniques rely on direct comparison between strains electrophoretic DNA patterns or DNA sequences. From an epidemiological point of view, this once more raises the recurring question of cut-off values to assign isolates to a same cluster for each of these techniques. This parameter is critical and should be described in every study dealing with the problematic at hands to allow readers to compare study results and make their own opinion on the relatedness of isolates. Ideally, for a given technique, a consensus cut-off should be reached and used by all works employing this method. This was partly the case for PFGE in the 2000s–2010s era with the criteria proposed by Tenover et al. [57], which were quite readily used by most investigators even though they were found too discriminatory by others [30]. The issue of cut-off values remains topical with WGS. As explained above, the number of SNPs indicative of distinct bacterial clones can vary from one species to another, depending on the estimated genome mutation rate for each species. This piece of information is therefore crucial for the reader. In this systematic review, only 57.8% on the papers actually mentioned (directly or indirectly) a cut-off value. This item was included on the checklist created to overcome another hurdle in this literature search, i.e., the lack of a tool for quality/risk of bias assessment suitable for our purpose. In addition to the study design and the reporting of cut-off values, we also included in this checklist, the completeness of the undertaken molecular investigation (i.e., were all isolates subjected to molecular typing), the inclusion of an outlier isolate/strain as an external control and the precise reporting of positivity for environmental and clinical samples. As for MLST, the cut-off value might not be the most suitable criterion but rather the precise identification of the scheme used to attribute an isolate to a given ST. Indeed, previous reports have shown discrepancies between MLST schemes available for a same bacterial species [68], which could lead to errors when comparing results obtained in papers using different MLST schemes.

Molecular techniques gave results in agreement with a possible patient-to-sink contamination in about two-third of the reports included in this work. This result can either be seen as over- or underestimated. On the one hand, the publication bias toward positive results might lead to an overestimation of this proportion. On the other hand, one could argue that it is not because sampling did not retrieve the epidemic clone(s) from the environment that it was not present, hence an underestimation of this contamination route. Indeed, enrichment of environmental samples was not performed and/or reported in most of the papers studied. Also, incubation times of up to 10 days have been reported as necessary to yield positive cultures from environmental samples [69], which is longer than incubation

times employed in most of the included studies. Only 17.8% (8/45) of the studies provided direct evidence of sink-to-patient transmission. The main hurdle to reach this conclusion was a lack of positivity timeline reporting in most studies. Indeed, along with how isolates are attributed to a same clone, the chronology of isolation is another fundamental criterion to judge the transmission direction. To these eight studies, two case-control studies could be added where staying in an ICU room with a contaminated sink/sink drain was identified as a risk factor for acquisition of the given MDR bacterial strain [34,43]. Most of the time, the sink-to-patient transmission is indirectly advocated for by the success of mitigation strategies such as (i) tap water-free or waterless care [26,54], (ii) sink drain decontamination with acetic acid or bleach [26,31,50], (iii) replacement of contaminated sinks/sink drains [21,26,30,50], (iv) implementation of self-disinfecting sink drains to get rid of bacterial contaminations [70,71], and (v) removal of sinks from ICUs [72].

Finally, the use of more discriminatory molecular biology techniques could remodel the landscape of evidence gathered for sink/sink drains as a possible reservoir for outbreaks of MDR bacteria in ICU. The percentage of studies successfully linking environmental isolates with clinical ones has indeed been reduced from 80% using PFGE to 50% using WGS. This decrease is not statistically significant ($p = 0.1155$, Fisher's exact test) as is but could become so with the addition of results from future studies using WGS to discriminate bacterial clones.

To conclude, the molecular biology evidence gathered in this review quite clearly underlines that ICU sink/sink drains can be contaminated by MDR bacteria originating from patients. Whether they can serve as a reservoir for further dissemination to other patients is less firmly established as most retrieved studies were carried out retrospectively and/or did not provide a complete and precise timeline for positive isolates. Future reports dealing with this topic should strive to provide the reader with this pivotal piece of information. It could for example take the shape of a detailed calendar including the retrieval date of each isolate, be it environmental or clinical. Along with the cut-off value used for discriminating bacterial isolates, whatever the technique used, this would help in comparing results from various origins and get a sharper and more solid image on the wet environment-to-patient transmission in ICUs.

Author Contributions: Conceptualization, M.C. and C.M.; methodology, C.M.; validation, M.C. and C.M.; formal analysis, M.C. and C.M.; writing—original draft preparation, M.C. and C.M.; writing—review and editing, M.C. and C.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Strich, J.R.; Kadri, S.S. Difficult-to-treat antibiotic-resistant Gram-negative pathogens in the intensive care unit: Epidemiology, outcomes, and treatment. *Semin. Respir. Crit. Care Med.* **2019**, *40*, 419–434. [CrossRef]
2. Kollef, M.H.; Torres, A.; Shorr, A.F.; Martin-Loeches, I.; Micek, S.T. Nosocomial infection. *Crit. Care Med.* **2021**, *49*, 169–187. [CrossRef] [PubMed]
3. Wang, L.; Du, K.N.; Zhao, Y.L.; Yu, Y.J.; Sun, L.; Jiang, H.B. Risk factors of nosocomial infection for infants in neonatal intensive care units: A systematic review and meta-analysis. *Med. Sci. Monit.* **2019**, *25*, 8213–8220. [CrossRef] [PubMed]
4. Palacios-Baena, Z.R.; Giannella, M.; Manissero, D.; Rodríguez-Baño, J.; Viale, P.; Lopes, S.; Wilson, K.; McCool, R.; Longshaw, C. Risk factors for carbapenem-resistant Gram-negative bacterial infections: A systematic review. *Clin. Microbiol. Infect.* **2021**, *27*, 228–235. [CrossRef] [PubMed]
5. De Waele, J.J.; Boelens, J.; Leroux-Roels, I. Multidrug-resistant bacteria in ICU: Fact or myth. *Curr. Opin. Anaesthesiol.* **2020**, *33*, 156–161. [CrossRef] [PubMed]

6. Brown, C.K.; Mitchell, A.H. Realigning the conventional routes of transmission: An improved model for occupational exposure assessment and infection prevention. *J. Hosp. Infect.* **2020**, *105*, 17–23. [CrossRef] [PubMed]
7. Boyce, J.M.; Pittet, D.; Healthcare Infection Control Practices Advisory Committee; Society for Healthcare Epidemiology of America; Association for Professionals in Infection Control; Infectious Diseases Society of America Hand Hygiene Task Force. Guideline for Hand Hygiene in Health-Care Settings: Recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. *Infect. Control Hosp. Epidemiol.* **2002**, *23*, S3–S40. [CrossRef]
8. Peters, A.; Schmid, M.N.; Parneix, P.; Lebowitz, D.; De Kraker, M.; Sauser, J.; Zingg, W.; Pittet, D. Impact of environmental hygiene interventions on healthcare-associated infections and patient colonization: A systematic review. *Antimicrob. Resist. Infect. Control* **2022**, *11*, 38. [CrossRef]
9. Eveillard, M.; Lemarié, C.; Legeay, C.; Ramont, C.; Onillon, L.; Corre, M.; Lasocki, S. Contamination of sink drains with carbapenemase-producing *Enterobacteriaceae* in intensive care units: A concern but don't worry so much! *J. Hosp. Infect.* **2019**, *103*, 475–477. [CrossRef]
10. Kearney, A.; Boyle, M.A.; Curley, G.F.; Humphreys, H. Preventing infections caused by carbapenemase-producing bacteria in the intensive care unit—Think about the sink. *J. Crit. Care* **2021**, *66*, 52–59. [CrossRef]
11. McGeer, A.; Low, D.E.; Penner, J.; Ng, J.; Goldman, C.; Simor, A.E. Use of molecular typing to study the epidemiology of *Serratia marcescens*. *J. Clin. Microbiol.* **1990**, *28*, 55–58. [CrossRef] [PubMed]
12. Page, M.J.; McKenzie, J.E.; Bossuyt, P.M.; Boutron, I.; Hoffmann, T.C.; Mulrow, C.D.; Shamseer, L.; Tetzlaff, J.M.; Akl, E.A.; Brennan, S.E.; et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ* **2021**, *372*, n71. [CrossRef]
13. Döring, G.; Hörz, M.; Ortelt, J.; Grupp, H.; Wolz, C. Molecular epidemiology of *Pseudomonas aeruginosa* in an intensive care unit. *Epidemiol. Infect.* **1993**, *110*, 427–436. [CrossRef] [PubMed]
14. Zhou, Z.; Hu, B.; Gao, X.; Bao, R.; Chen, M.; Li, H. Sources of sporadic *Pseudomonas aeruginosa* colonizations/infections in surgical ICUs: Association with contaminated sink trap. *J. Infect. Chemother.* **2016**, *22*, 450–455. [CrossRef] [PubMed]
15. Qiao, F.; Wei, L.; Feng, Y.; Ran, S.; Zheng, L.; Zhang, Y.; Xiang, Q.; Liu, Y.; Wu, X.; Duan, X.; et al. Handwashing sink contamination and carbapenem-resistant *Klebsiella* infection in the intensive care unit: A prospective multicenter study. *Clin. Infect. Dis.* **2020**, *71*, S379–S385. [CrossRef]
16. Lemarié, C.; Legeay, C.; Mahieu, R.; Moal, F.; Ramont, C.; Kouatchet, A.; Eveillard, M. Long-term contamination of sink drains by carbapenemase-producing Enterobacterales in three intensive care units: Characteristics and transmission to patients. *J. Hosp. Infect.* **2021**, *112*, 16–20. [CrossRef] [PubMed]
17. Milisavljevic, V.; Wu, F.; Larson, E.; Rubenstein, D.; Ross, B.; Drusin, L.M.; Della-Latta, P.; Saiman, L. Molecular epidemiology of *Serratia marcescens* outbreaks in two neonatal intensive care units. *Infect. Control Hosp. Epidemiol.* **2004**, *25*, 719–721. [CrossRef] [PubMed]
18. Wagenlehner, F.M.; MacKenzie, F.M.; Forbes, K.J.; Gould, I.M. Molecular epidemiology and antibiotic resistance of *Enterobacter* spp. from three distinct populations in Grampian, UK. *Int. J. Antimicrob. Agents* **2002**, *20*, 419–425. [CrossRef]
19. Wendel, A.F.; Kolbe-Busch, S.; Ressina, S.; Schulze-Röbbecke, R.; Kindgen-Milles, D.; Lorenz, C.; Pfeffer, K.; MacKenzie, C.R. Detection and termination of an extended low-frequency hospital outbreak of GIM-1-producing *Pseudomonas aeruginosa* ST111 in Germany. *Am. J. Infect. Control* **2015**, *43*, 635–639. [CrossRef]
20. Blanc, D.S.; Senn, L.; Federli, I.; Giannoni, E.; Legault, F.; Roth-Kleiner, M.; Grandbastien, B. Whole genome sequencing sustains cross-transmission of *Pseudomonas aeruginosa* in a neonatal intensive care unit during an outbreak. Abstract ICPIC 2021 O07. *Antimicrob. Resist. Infect. Control* **2021**, *10* (Suppl. S1), 130. [CrossRef]
21. Longtin, Y.; Troillet, N.; Touveneau, S.; Boillat, N.; Rimensberger, P.; Dharan, S.; Gervaix, A.; Pittet, D.; Harbarth, S. *Pseudomonas aeruginosa* outbreak in a pediatric intensive care unit linked to a humanitarian organization residential center. *Pediatr. Infect. Dis. J.* **2010**, *29*, 233–237. [CrossRef] [PubMed]
22. Orr, K.; Gould, F.K.; Sisson, P.R.; Lightfoot, N.F.; Freeman, R.; Burdess, D. Rapid inter-strain comparison by pyrolysis mass spectrometry in nosocomial infection with *Xanthomonas maltophilia*. *J. Hosp. Infect.* **1991**, *17*, 187–195. [CrossRef]
23. Kerr, J.R.; Moore, J.E.; Curran, M.D.; Graham, R.; Webb, C.H.; Lowry, K.G.; Murphy, P.G.; Wilson, T.S.; Ferguson, W.P. Investigation of a nosocomial outbreak of *Pseudomonas aeruginosa* pneumonia in an intensive care unit by random amplification of polymorphic DNA assay. *J. Hosp. Infect.* **1995**, *30*, 125–131. [CrossRef]
24. Verweij, P.E.; Meis, J.F.; Christmann, V.; Van der Bor, M.; Melchers, W.J.; Hilderink, B.G.; Voss, A. Nosocomial outbreak of colonization and infection with *Stenotrophomonas maltophilia* in preterm infants associated with contaminated tap water. *Epidemiol. Infect.* **1998**, *120*, 251–256. [CrossRef]
25. Su, L.H.; Leu, H.S.; Chiu, Y.P.; Chia, J.H.; Kuo, A.J.; Sun, C.F.; Lin, T.Y.; Wu, T.L. Molecular investigation of two clusters of hospital-acquired bacteraemia caused by multi-resistant *Klebsiella pneumoniae* using pulsed-field gel electrophoresis and infrequent restriction site PCR. *J. Hosp. Infect.* **2000**, *46*, 110–117. [CrossRef]
26. Hoque, S.N.; Graham, J.; Kaufmann, M.E.; Tabaqchali, S. *Chryseobacterium (Flavobacterium) meningosepticum* outbreak associated with colonization of water taps in a neonatal intensive care unit. *J. Hosp. Infect.* **2001**, *47*, 188–192. [CrossRef]
27. Dijk, Y.; Bik, E.M.; Hochstenbach-Vernooij, S.; Vlist, G.J.; Savelkoul, P.H.; Kaan, J.A.; Diepersloot, R.J. Management of an outbreak of *Enterobacter cloacae* in a neonatal unit using simple preventive measures. *J. Hosp. Infect.* **2002**, *51*, 21–26. [CrossRef]

28. Kac, G.; Podglajen, I.; Vaupré, S.; Colardelle, N.; Buu-Hof, A.; Gutmann, L. Molecular epidemiology of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* isolated from environmental and clinical specimens in a cardiac surgery intensive care unit. *Infect. Control Hosp. Epidemiol.* **2004**, *25*, 852–855. [CrossRef]
29. Maragakis, L.L.; Winkler, A.; Tucker, M.G.; Cosgrove, S.E.; Ross, T.; Lawson, E.; Carroll, C.; Perl, T.M. Outbreak of multidrug-resistant *Serratia marcescens* infection in a neonatal intensive care unit. *Infect. Control Hosp. Epidemiol.* **2008**, *29*, 418–423. [CrossRef]
30. Hota, S.; Hirji, Z.; Stockton, K.; Lemieux, C.; Dedier, H.; Wolfaardt, G.; Gardam, M.A. Outbreak of multidrug-resistant *Pseudomonas aeruginosa* colonization and infection secondary to imperfect intensive care unit room design. *Infect. Control Hosp. Epidemiol.* **2009**, *30*, 25–33. [CrossRef]
31. La Forgia, C.; Franke, J.; Hacek, D.M.; Thomson, R.B., Jr.; Robicsek, A.; Peterson, L.R. Management of a multidrug-resistant *Acinetobacter baumannii* outbreak in an intensive care unit using novel environmental disinfection: A 38-month report. *Am. J. Infect. Control* **2010**, *38*, 259–263. [CrossRef]
32. Lucero, C.A.; Cohen, A.L.; Trevino, I.; Rupp, A.H.; Harris, M.; Forkan-Kelly, S.; Noble-Wang, J.; Jensen, B.; Shams, A.; Arduino, M.J.; et al. Outbreak of *Burkholderia cepacia* complex among ventilated pediatric patients linked to hospital sinks. *Am. J. Infect. Control* **2011**, *39*, 775–778. [CrossRef] [PubMed]
33. Guleri, A.; Palmer, R.; Jackson, P.; Przybylo, M.; Sharma, R.; Peel, A.; Bird, M.; Moorhouse, L.; Clitheroe, S.; King, S.; et al. Outbreak report of multidrug-resistant *Pseudomonas aeruginosa* [MDR-PA] in a cardiac intensive care unit at a Lancashire cardiac centre. *Clin. Microbiol. Infect.* **2012**, *18* (Suppl. S3), 13.
34. Diederen, B.M.W.; Hattink-Malipaard, C.J.R.; Vloemans, A.F.P.M.; Hene, I.; Euser, S.M.; Van Der Reijden, W.A.; Van Ess, I.F. A prolonged hospital outbreak with metallo-betalactamase producing *Pseudomonas aeruginosa* in a burn centre and intensive care unit linked to an environmental reservoir. *Clin. Microbiol. Infect.* **2012**, *18* (Suppl. S3), 14.
35. Maltezou, H.C.; Tryfinopoulou, K.; Katerelos, P.; Ftika, L.; Pappa, O.; Tseroni, M.; Kostis, E.; Kostalos, C.; Prifti, H.; Tzanetou, K.; et al. Consecutive *Serratia marcescens* multiclone outbreaks in a neonatal intensive care unit. *Am. J. Infect. Control* **2012**, *40*, 637–642. [CrossRef] [PubMed]
36. Lowe, C.; Willey, B.; O'Shaughnessy, A.; Lee, W.; Lum, M.; Pike, K.; Larocque, C.; Dedier, H.; Dales, L.; Moore, C.; et al. Mount Sinai Hospital Infection Control Team. Outbreak of extended-spectrum β-lactamase-producing *Klebsiella oxytoca* infections associated with contaminated handwashing sinks (1). *Emerg. Infect. Dis.* **2012**, *18*, 1242–1247. [CrossRef] [PubMed]
37. Starlander, G.; Melhus, Å. Minor outbreak of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* in an intensive care unit due to a contaminated sink. *J. Hosp. Infect.* **2012**, *82*, 122–124. [CrossRef] [PubMed]
38. Kotsanas, D.; Wijesooriya, W.R.P.L.I.; Korman, T.M.; Gillespie, E.E.; Wright, L.; Snook, K.; Williams, N.; Bell, J.M.; Li, H.Y.; Stuart, R.L. "Down the drain": Carbapenem-resistant bacteria in intensive care unit patients and handwashing sinks. *Med. J. Aust.* **2013**, *198*, 267–269. [CrossRef]
39. Landelle, C.; Legrand, P.; Lesprit, P.; Cizeau, F.; Ducellier, D.; Gouot, C.; Bréhaut, P.; Soing-Altrach, S.; Girou, E.; Brun-Buisson, C. Protracted outbreak of multidrug-resistant *Acinetobacter baumannii* after intercontinental transfer of colonized patients. *Infect. Control Hosp. Epidemiol.* **2013**, *34*, 119–124. [CrossRef]
40. Vergara-López, S.; Domínguez, M.C.; Conejo, M.C.; Pascual, Á.; Rodríguez-Baño, J. Wastewater drainage system as an occult reservoir in a protracted clonal outbreak due to metallo-β-lactamase-producing *Klebsiella oxytoca*. *Clin. Microbiol. Infect.* **2013**, *19*, E490–E498. [CrossRef]
41. Davis, R.J.; Jensen, S.O.; Van Hal, S.; Espedido, B.; Gordon, A.; Farhat, R.; Chan, R. Whole genome sequencing in real-time investigation and management of a *Pseudomonas aeruginosa* outbreak on a neonatal intensive care unit. *Infect. Control Hosp. Epidemiol.* **2015**, *36*, 1058–1064. [CrossRef] [PubMed]
42. Umezawa, K.; Asai, S.; Ohshima, T.; Iwashita, H.; Ohashi, M.; Sasaki, M.; Kaneko, A.; Inokuchi, S.; Miyachi, H. Outbreak of drug-resistant *Acinetobacter baumannii* ST219 caused by oral care using tap water from contaminated hand hygiene sinks as a reservoir. *Am. J. Infect. Control* **2015**, *43*, 1249–1251. [CrossRef] [PubMed]
43. Salm, F.; Deja, M.; Gastmeier, P.; Kola, A.; Hansen, S.; Behnke, M.; Gruhl, D.; Leistner, R. Prolonged outbreak of clonal MDR *Pseudomonas aeruginosa* on an intensive care unit: Contaminated sinks and contamination of ultra-filtrate bags as possible route of transmission? *Antimicrob. Resist. Infect. Control* **2016**, *5*, 53. [CrossRef] [PubMed]
44. De Geyter, D.; Blommaert, L.; Verbraeken, N.; Sevenois, M.; Huyghens, L.; Martini, H.; Covens, L.; Piérard, D.; Wybo, I. The sink as a potential source of transmission of carbapenemase-producing *Enterobacteriaceae* in the intensive care unit. *Antimicrob. Resist. Infect. Control.* **2017**, *6*, 24. [CrossRef]
45. Herruzo, R.; Ruiz, G.; Gallego, S.; Diez, J.; Sarria, A.; Omeñaca, F. VIM-*Klebsiella oxytoca* outbreak in a neonatal intensive care unit. This time it wasn't the drain. *J. Prev. Med. Hyg.* **2017**, *58*, E302–E307. [CrossRef]
46. Cantero, M.; Parra, L.M.; Muñez, E.; Iranzo, R.; Sánchez-Romero, M.I.; Oteo, J.; Asensio, A. A cluster of *Chryseobacterium indologenes* cases related to drainage water in intensive care units. *Infect. Control. Hosp. Epidemiol.* **2018**, *39*, 997–999. [CrossRef]
47. Regev-Yochay, G.; Smollan, G.; Tal, I.; Pinas Zade, N.; Haviv, Y.; Nudelman, V.; Gal-Mor, O.; Jaber, H.; Zimlichman, E.; Keller, N.; et al. Sink traps as the source of transmission of OXA-48-producing *Serratia marcescens* in an intensive care unit. *Infect. Control Hosp. Epidemiol.* **2018**, *39*, 1307–1315. [CrossRef]

48. Martischang, R.; Sauvan, V.; Leo, S.; Lazarevic, V.; Chraïti, M.N.; Martin, Y.; Soule, H.; Schrenzel, J.; Koyluk Tomsuk, Z.; Pugin, J.; et al. "behind the sinks": Endemic *Serratia marcescens* in a Swiss intensive care unit (ICU). *Antimicrob. Res. Infect. Control* **2019**, *8* (Suppl. S1), 148. [CrossRef]
49. Avaness, M.; Salt, N.; Maze dit Mieusement, L.; Marchand-Senecal, X.; Leis, J. Role of sinks as an environmental reservoir in CPE outbreak in a burn intensive care unit. *Am. J. Infect. Control* **2020**, *48*, S39. [CrossRef]
50. Jung, J.; Choi, H.S.; Lee, J.Y.; Ryu, S.H.; Kim, S.K.; Hong, M.J.; Kwak, S.H.; Kim, H.J.; Lee, M.S.; Sung, H.; et al. Outbreak of carbapenemase-producing *Enterobacteriaceae* associated with a contaminated water dispenser and sink drains in the cardiology units of a Korean hospital. *J. Hosp. Infect.* **2020**, *104*, 476–483. [CrossRef] [PubMed]
51. Yeo, K.T.; Octavia, S.; Lim, K.; Lin, C.; Lin, R.; Thoon, K.C.; Tee, N.W.S.; Yung, C.F. *Serratia marcescens* in the neonatal intensive care unit: A cluster investigation using molecular methods. *J. Infect. Public Health* **2020**, *13*, 1006–1011. [CrossRef] [PubMed]
52. De Man, T.J.B.; Yaffee, A.Q.; Zhu, W.; Batra, D.; Alyanak, E.; Rowe, L.A.; McAllister, G.; Moulton-Meissner, H.; Boyd, S.; Flinchum, A.; et al. Multispecies outbreak of Verona integron-encoded metallo-ß-lactamase-producing multidrug resistant bacteria driven by a promiscuous incompatibility group A/C2 plasmid. *Clin. Infect. Dis.* **2021**, *72*, 414–420. [CrossRef]
53. Gideskog, M.; Welander, J.; Melhus, Å. Cluster of *Stenotrophomonas maltophilia* among patients with respiratory tract infections at an intensive care unit. *Infect. Prev. Pract.* **2020**, *2*, 100097. [CrossRef] [PubMed]
54. Catho, G.; Martischang, R.; Boroli, F.; Chraïti, M.N.; Martin, Y.; Koyluk Tomsuk, Z.; Renzi, G.; Schrenzel, J.; Pugin, J.; Nordmann, P.; et al. Outbreak of *Pseudomonas aeruginosa* producing VIM carbapenemase in an intensive care unit and its termination by implementation of waterless patient care. *Crit. Care* **2021**, *25*, 301. [CrossRef] [PubMed]
55. Bourdin, T.; Monnier, A.; Benoit, M.È.; Bédard, E.; Prévost, M.; Quach, C.; Déziel, E.; Constant, P. A High-throughput short sequence typing scheme for *Serratia marcescens* pure culture and environmental DNA. *Appl. Environ. Microbiol.* **2021**, *87*, e0139921. [CrossRef] [PubMed]
56. Tenover, F.C.; Arbeit, R.D.; Goering, R.V.; Mickelsen, P.A.; Murray, B.E.; Persing, D.H.; Swaminathan, B. Interpreting chromosomal DNA restriction patterns produced by pulse-field gel electrophoresis: Criteria for bacterial strain typing. *J. Clin. Microbiol.* **1995**, *33*, 2233–2239. [CrossRef]
57. Hopman, J.; Meijer, C.; Kenters, N.; Coolen, J.P.M.; Ghamati, M.R.; Mehtar, S.; Van Crevel, R.; Morshuis, W.J.; Verhagen, A.F.T.M.; Van den Heuvel, M.M.; et al. Risk assessment after a severe hospital-acquired infection associated with carbapenemase-producing *Pseudomonas aeruginosa*. *JAMA Netw. Open* **2019**, *2*, e187665. [CrossRef]
58. Grundmann, H.; Kropec, A.; Hartung, D.; Berner, R.; Daschner, F. *Pseudomonas aeruginosa* in a neonatal intensive care unit: Reservoirs and ecology of the nosocomial pathogen. *J. Infect. Dis.* **1993**, *168*, 943–947. [CrossRef]
59. Kim, S.H.; Kim, G.R.; Kim, E.Y.; Jeong, J.; Kim, S.; Shin, J.H. Carbapenemase-producing enterobacterales from hospital environment and their relation to those from patient specimens. *J. Infect. Public Health* **2022**, *15*, 241–244. [CrossRef]
60. Smolders, D.; Hendriks, B.; Rogiers, P.; Mul, M.; Gordts, B. Acetic acid as a decontamination method for ICU sink drains colonized by carbapenemase-producing Enterobacteriaceae and its effect on CPE infections. *J. Hosp. Infect.* **2019**, *102*, 82–88. [CrossRef]
61. Döring, G.; Ulrich, M.; Müller, W.; Bitzer, J.; Schmidt-Koenig, L.; Münst, L.; Grupp, H.; Wolz, C.; Stern, M.; Botzenhart, K. Generation of *Pseudomonas aeruginosa* aerosols during handwashing from contaminated sink drains, transmission to hands of hospital personnel, and its prevention by use of a new heating device. *Zentralbl. Hyg. Umweltmed.* **1991**, *191*, 494–505. [PubMed]
62. Franco, L.C.; Tanner, W.; Ganim, C.; Davy, T.; Edwards, J.; Donlan, R. A microbiological survey of handwashing sinks in the hospital built environment reveals differences in patient room and healthcare personnel sinks. *Sci. Rep.* **2020**, *10*, 8234. [CrossRef] [PubMed]
63. Love, N.K.; Pichon, B.; Padfield, S.; Hughes, G.J. A persistent recurring cluster of meticillin-resistant *Staphylococcus aureus* (MRSA) colonizations in a special care baby unit: A matched case-control study. *J. Hosp. Infect.* **2020**, *106*, 774–781. [CrossRef]
64. Madera, S.; McNeil, N.; Serpa, P.H.; Kamm, J.; Pak, C.; Caughell, C.; Nichols, A.; Dynerman, D.; Li, L.M.; Sanchez-Guerrero, E.; et al. Prolonged silent carriage, genomic virulence potential and transmission between staff and patients characterize a neonatal intensive care unit (NICU) outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA). *Infect. Control Hosp. Epidemiol.* **2022**, 1–7. [CrossRef]
65. McKew, G.; Ramsperger, M.; Cheong, E.; Gottlieb, T.; Sintchenko, V.; O'Sullivan, M. Hospital MRSA outbreaks: Multiplex PCR-reverse line blot binary typing as a screening method for WGS, and the role of the environment in transmission. *Infect. Dis. Health* **2020**, *25*, 268–276. [CrossRef] [PubMed]
66. Kampmeier, S.; Tönnies, H.; Correa-Martinez, C.L.; Mellmann, A.; Schwierzeck, V. A nosocomial cluster of vancomycin resistant enterococci among COVID-19 patients in an intensive care unit. *Antimicrob. Resist. Infect. Control* **2020**, *9*, 154. [CrossRef]
67. Otter, J.A.; Vickery, K.; Walker, J.T.; DeLancey Pulcini, E.; Stoodley, P.; Goldenberg, S.D.; Salkeld, J.A.G.; Chewins, J.; Yezli, S.; Edgeworth, J.D. Surface-attached cells, biofilms and biocide susceptibility: Implications for hospital cleaning and disinfection. *J Hosp Infect* **2015**, *89*, 16–27. [CrossRef]
68. Gaiarsa, S.; Batisti Biffignandi, G.; Esposito, E.P.; Castelli, M.; Jolley, K.A.; Brisse, S.; Sassera, D.; Zarrilli, R. Comparative analysis of the two *Acinetobacter baumannii* multilocus sequence typing (MLST) schemes. *Front. Microbiol.* **2019**, *10*, 930. [CrossRef]
69. Lalancette, C.; Charron, D.; Laferrière, C.; Dolcé, P.; Déziel, E.; Prévost, M.; Bédard, E. Hospital drains as reservoirs of *Pseudomonas aeruginosa*: Multiple-locus variable-number of tandem repeats analysis genotypes recovered from faucets, sink surfaces and patients. *Pathogens* **2017**, *6*, 36. [CrossRef]

70. Fusch, C.; Pogorzelski, D.; Main, C.; Meyer, C.L.; El Helou, S.; Mertz, D. Self-disinfecting sink drains reduce the *Pseudomonas aeruginosa* bioburden in a neonatal intensive care unit. *Acta Paediatr.* **2015**, *104*, e344–e349. [CrossRef]
71. Mathers, A.J.; Vegesana, K.; German Mesner, I.; Barry, K.E.; Pannone, A.; Baumann, J.; Crook, D.W.; Stoesser, N.; Kotay, S.; Carroll, J.; et al. Intensive care uUnit wastewater interventions to prevent transmission of multispecies *Klebsiella pneumoniae* carbapenemase-producing organisms. *Clin. Infect. Dis.* **2018**, *67*, 171–178. [CrossRef] [PubMed]
72. Shaw, E.; Gavaldà, L.; Càmara, J.; Gasull, R.; Gallego, S.; Tubau, F.; Granada, R.M.; Ciercoles, P.; Dominguez, M.A.; Mañez, R.; et al. Control of endemic multidrug-resistant Gram-negative bacteria after removal of sinks and implementing a new water-safe policy in an intensive care unit. *J. Hosp. Infect.* **2018**, *98*, 275–281. [CrossRef] [PubMed]