Original Research Article

Yeast and Filamentous Fungi in the Jerry cans of water vendors (Meruwa) in Toru – Orua, Sagbama. Bayelsa State

Abstract:

Access to safe portable water have long constituted a challenge to man. Majority of the people in Toru - Orua, Bayelsa State depend on water vendors for their water supply and hence the need to examine the yeast and filamentous fungi associated with the Jerrycan used by these water vendors (Meruwa). Culture dependent method was used to investigate the microbial quality of the vendors jerry can and the water from three different locations. The result from the study showed that the swab samples in location 1 recorded Candida spp (70%), Aspergillus sp (16%) Geotrichum sp (9%) and Rhizopus sp (5%). in location 2, swab sample showed three different genera that showed Candida spp recording the highest percentage (66%) Aspergillus sp (19%) and Fusarium spp (15%). Candida spp was observed to be the most dominant yeast specie associated with the Jerry cans while Aspergillus was the most dominant in the water sample. The prevalence of the yeast and filamentoud fungi in the Jerry cans used by water vendors may result to illnesses, therefore the Jerry cans ought to be properly and regularly cleaned.

Keywords: Yeast, Filamentous Fungi, Jerry can

Introduction

Water is one of the most important resources for the survival of human beings, humans use water for numerous purposes such as drinking, washing, cooking and bathing. There has never been any doubt that water is an essential and basic requirement for both humans and animals. In fact, in its pure state water is acclaimed key to health and general contention is that, water is more basic than all other essential things in life (Singh et al., 2012). Thus the supply of hygienic, safe and clean water is necessary for the health and survival of humans. This suggests that man would require an unwavering and accessible supply of water, which forms a major component of the protoplasm and provides an essential requirement for vital physiological and biochemical processes. The vital role of water was emphasized in the work of Muyi (2008), which posited that "man can

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go without food for twenty-eight days but only three days without water, and two third of a person's water consumption per day is through food while one third is obtained through drinking". To meet the demand of water by human populations, there exist countless sources through which good water can be obtained. They are; Groundwater, Surface water and Rainwater.

On a global scale, groundwater represents the world's prevalent and most important source of fresh portable water (Muyi, 2008). Freshwater available for human consumption represents only 0.6% of global water supplies stored in glaciers, running surface water and ground water (Wurzbacher et al., 2011). Depending on the geological features of the area, either groundwater or surface water is used as a primary source to produce tap water (DEFRA 2011; Gray, 2014). Groundwater affords potable water to an estimated 1.5billion people worldwide daily, and has proven to be the most unswerving resource for meeting rural water demands in Sub – Saharan Africa (Akpoveta, 2011). Consquently, people have resort to ground water sources such as boreholes as an alternative water resource. Sadly boreholes are usually quite expensive to drill and operate, so a vast majority of people residing in Nigeria relies on buying water from commercial boreholes. The borehole water is stored in drums, gallons and other suitable containers in homes after purchase. Alternatively, people depend on the water supplied by water vendors commonly called "Meruwa". The water vendors usually supply borehole water to houses with gallons carried about in big trucks, and they provide a good water supply service to many homes. However, there are some hick ups associated with the distribution of water, by water vendors (Meruwa). There is the issue of water quality and has raised much concerns to the consumers.

One of the greatest concerns for the water consumers with respect to the quality of drinking water is contamination by pathogenic microorganisms. Certain microorganisms, including various bacteria, viruses and parasites are well known water contaminants of which several may lead to the occurrence of a waterborne

disease and epidemic. Bacteria are the most frequently studied group of microorganisms with respect to the quality of drinking water (Mara and Horan, 2006). In the past, fungi were infrequently considered when considering pathogenic microorganisms in water. However, these days fungi now receive an increased focus as drinking water contaminants and have continued to raise concerns for various reasons. For example, some fungi growing in drinking water resources cause problems in the taste and the odor of water.

Fungi are a diverse group of organisms belonging to the kingdom Eumycota. This kingdom comprises of five phyla namely; Ascomycota, Basidiomycota, Zygomycota, Chytridiomycota and Glomeromycota (Kirk et al., 2001). As a practical approach to classification, fungi have been divided into groups such as the filamentous fungi, also called moulds, the yeasts, and the mushrooms. Some of these fungi are primarily adapted to aquatic environments and will therefore naturally be found in water. These fungi are zoosporic, and many belong to the phyl Chytridiomycota. Fungi belonging to the other phyla in Eumycota are primarily adapted to terrestrial environments. They are present in soil, organic material and air and anything in contact with air (Kirk et al., 2001). However, these fungi can also enter drinking water from various locations.

Yeasts and filamentous fungi have long been implicated as contaminants of drinking water (Kirk et al., 2001). Yeast are widespread in terrestrial, aquatic and aerial environments and their distribution, frequency and metabolic characteristics have been found to be governed by the existing environmental conditions. They are ecologically flexible, which allows them to tolerate a wide range of salinities, environmental temperatures, oxygen saturation levels, and acidities in the surrounding medium (Boguslawska – was and Dabrowski, 2001). Yeast include *Candida albicans, Cryptococcus tropicalis, Rhodotorula spp.* In most cases, the dominant species contaminating water are from the *Rhodotorula, Candida* and *Cryptococcus* genera (Kirk et al., 2001).

Filamentous fungi are a diverse group of heterotrophic microorganisms that are medically and agriculturally important and are also widely used in the production of food, beverages, antibiotics, enzymes and organic acids and in biomass conversion. However, they are also serious human pathogens, especially to immune – compromised patients and have been reported to account for up to 40% of deaths from hospital acquired infections (Muthuvijayan et el., 2004). Filamentous fungi include *Alternaria spp.*, *Aspergillus spp.*, *Fusarium spp.*, *Rhizopus spp*., Etc.

Many of the fungi that have been isolated from treating drinking water are known to be pathogenic particularly *Aspergillus* and *Candida*. Fungi have also been linked to allergic disease, including worsening of asthma symptoms, hypersensitivity pneumonitis and skin irritation. Fungi known to provoke allergic responses in susceptible individuals, such as *Alternaria spp*., *Aspergillus spp*., *Cladosporium spp*., and *Penicillum spp*., have been isolated from drinking water. Symptoms have risen due to exposure when showering, bathing or from exposure to water – damaged buildings. Some fungi, including *Penicillum spp*., *Aspergillus spp*., *Fusariam spp*., and *Claviceps spp* are known to produce mycotoxins such as patulin, aflatoxins and zearalenone.

It is important to note that a number of factors may influence the types and number of yeasts and filamentous fungi present in water. Fungi are more likely to be isolated in drinking water derived from surface water than from drinking water derived from groundwater. This may be due to the larger amounts of organic matter in surface water. Also, differences in acidity and calcium content may also account for some of the variation. Humidity, temperature, water potential and P^H, water treatment, use of materials for water distribution systems and consequently the possibility of biofilm formation also have a critical influence on the growth and survival of fungi (De Bruin, 2008). Due to their tolerance of oligotrophic environments, some species of fungi are able to colonize drinking water distribution systems, which are typically low in

nutrients. Biofilms are an important habitat for fungi in drinking water. Their development is influenced by many factors including temperature, nutrient concentration, pipe material and water flow rate. The problem of biofilm formation is common in Jerry cans because it provides a favorable environment for their growth and survival. A number of treatment procedures are often used to eradicate microorganisms in drinking water, including fungi. Nevertheless, water treatment appears to reduce the number of fungi in water, without removing all of them.

The purpose of water treatment is to provide clean water that does not contain objectionable taste, odor or colour. All water produced in public water systems is required to achieve clean water quality, even though only about 1% of water produced is used for drinking and cooking. Clean and treated water should be accessible to all persons not only for an urban population where all the facilities and amenities are available but also for persons who live in remote and rural areas.

Fungi are accounted as a significant cause of water pollution due to having the ability to survive after filtration (Wurzbacher et al., 2011). Fungi have been reported as pollutant and contaminant of all types of water, like raw water, treated water and even distilled or bottled water. The presence of fungi in water have often been overlooked, but it may come as a chronic problem in drinking water and the formation of biofilms may aid other potential pathogenic microorganisms to increase in number or spread rapidly. It is thought that the threshold level for numbers of fungi that can cause problems may be around 10^2 - 10^3 CFU per litre.

Fungi can release sulfur compounds from the metabolic oxidation of substrates resulting to unpleasant taste and offensive odor of water. Many species of the genus fungi particularly *Aspergillus spp.* are found in water and can cause kidney problem, liver disorders, allergy, intensify the burn marks, otitis and increase risk of invasive infections (Rankovic, 2005; Goncalves et al., 2006).

The problems of fungi contamination of water are numerous. Worse, the use of jerry cans in water supply may further promote the growth of filamentous fungi and yeasts, it may promote the formation of biofilms which will serve as a source of nutrient and home to other harmful microorganisms. Thereby, putting hundreds to thousands of consumers at great risk.

MATERIALS AND METHODS

Sample Area

The sample area is part of the Toru – Orua community, in Sagbama Local Government Area, Bayelsa State. This study was conducted on the 16th of November, 2022 just after the perennial flooding. Over the course of the year, the temperature varied from 22^{0}C to 31^{0}C and rarely below 17^{0}C or above 32^{0}C.

Four samples each were collected from each of the jerry cans (2 swab and 2 water samples) from the two jerry cans at three different locations. For the study, the distance between each of the locations is between 1 to 2 kilometres away from each other.

Location 1: Angalabiri

Location 2: Ebedebiri

Location 3: A & K Road, Toru – Orua

Sample Collection

A total of 24 samples were collected on the 22nd of November, 2022 from the three different locations and in each of the locations, samples were collected

from two different jerry cans in a truck. Also from each of the jerry cans, two water samples and two swab samples were collected. The water in the jerry cans was all gotten from boreholes in the different locations. The water was aseptically collected with plastic containers and the orifice of the jerry cans was also swabbed. All collected samples were transported directly to the laboratory immediately after collection, with the original storage conditions been maintained using an ice pack container.

Sterilization of Materials

The media was sterilized for 15minutes at a temperature of 121^0C. Glassware were also sterilized at 121^0C for 15minutes using the autoclave while materials not suitable for autoclaving were sterilized by disinfecting thoroughly with 70% ethanol, the work bench was also thoroughly disinfected using 70% ethanol to avoid contamination.

Media Preparation

The first step in media preparation is to assemble the equipment and media.

The following culture media were used in this study;

- Sabour and dextrose agar: This is used for the isolation, cultivation and maintenance of non – pathogenic species of fungi and yeast.
- Yeast extract: This provides microoranisms and cell with essential nutrients such as viatamins, trace elements and growth factor.

  The culture media was prepared according to the manufacturers instruction.

Mycological Analysis

The cultivation and isolation of the filamentous and yeast associated with the water samples was done using culture dependent methods. 1ml of each of the water samples was introduced into a well-labeled sterile test tube containing 10ml of 0.85% of normal saline. The tubes were vigorously agitated to

dislodge the microbes associated with the surfaces of the swab samples into the saline solution. After which, test tubes containing 9ml of normal saline were set up in test tube racks and labeled. Tenfold serial dilution was done – 1ml of the inoculums from the original fungal stock (10ml of normal saline tube) was collected aseptically and transferred into the first dilution tube (10^{-2}). The samples were diluted four times in order to obtain an acceptable colony count. The tubes were covered swiftly with cotton wool to prevent the contamination of the samples.

Plating was done in triplicates with the second dilution tube (10^{-2}) using pour plate method. 1ml of the inoculums was aseptically collected with a syringe and was poured into the petri dishes. 20ml of nutrient medium was poured into the petri dishes were swirled gently to spread the inoculums evenly in the medium. The plates were allowed to set (solidify) and were inverted and thereafter incubated at room temperature for 5days. After the incubation time, the plates were observed for the number of colonies and colony morphology. The colonies were randomly selected and were picked off with sterile wire needle. The colonies were sub cultured on fresh SDA plates and yeast extract plates.

Morphological Identification of Fungi

The plates were examined for the morphological characteristics of the fungal colonies. The microscopic observation was aimed at determining the size, shape, growth and colour of the plate. This was done with a hand lens.

Microscopic Examination of Fungal Isolates

The examination and microscopic examination of fungal isolates requires the observation of microscopic features such as shape, size of hyphae, shape od sporangia, conidia, conidiophores and spores. Using a flamed inoculating needle, the edge of each colony is picked and slides of different cdolonies are

made, a drop of Lacto-phenol cotton blue stain is added to the slides and covered with cover slip and examine under the microscope using **×100 and ×400** magnification starting from third day of the culture. The microscopic characteristics observed were recorded accordingly.

Lacto Phenol Cotton Blue Staining Technique

Lacto phenol cotton blue wet mount that is most widely used in the preparation of slides for microscopic examination of fungi.

- A drop of 70% ethanol was placed on a clean microscopic glass slide
- The test fungal isolate was immersed in the drop of alcohol
- Two drops of Lacto – Phenol cotton blue was added
- The wet preparation was covered with a glass cover slip
- The wet preparation was examined using low power objective and thereafter, 40× objective

Results

The results for the enumeration of the fungal species associated with the Jerry cans of water vendors are shown in the table 1. below. The results show mean fungal counts obtained from the jerry can swab ranging from 1.1×10^3 to 5.6×10^3, while the mean counts obtained from the water sample ranged from 0.8×10^3 to 1.2×10^3. The swab samples are shown to record the highest fungal counts when compared to the water samples obtained from the Jerry cans.

Table 1: Enumeration of Fungal species on SDA

Samples	Mean	Cfu/g
1A	42	4.2×10^3
1B	11	1.1×10^3

2A	11	1.1×10^3
2B	56	5.6×10^3
3A	08	0.8×10^3
3B	12	1.2×10^3

KEY A:

1A: Location 1 Water sample

1B: Location 1 Swab sample

2A: Location 2 Water sample

2B: Location 2 Swab sample

3A: Location 3 Water sample

3B: Location 3 Swab sample

Table 2. below shows the results for the enumeration of fungal species on yeast extract agar. The mean fungal counts of the swab samples in the different locations ranged from 0.7×10^3 to 1.0×10^3, while the fungal counts obtained from the water samples ranged from 0.4×10^3 to 2.2×10^3. The swab samples are shown to record the highest fungal counts when compared to the water samples obtained from the Jerry cans.

Table 2: Enumeration of Fungal species on Yeast Extract Agar

Samples	Mean	Cfu/g
1A	42	4.2×10^3
1B	11	1.1×10^3
2A	11	1.1×10^3
2B	56	5.6×10

3A	08	0.8×10^3
3B	12	1.2×10^3

The identification of the fungal species associated with water vendor jerry cans in sample 1 is shown below. The fungal species were identified based on their microscopic and macroscopic features. Four different fungal genera were identified; *Aspergillus sp, Rhizopus spp, Candida spp, and Geotrichum spp*.

Table 3: Fungal isolates from Sample 1 (Water and Swab)

Macroscopic Features	Microscopic Features	Fungi Isolates
Colourless, finely Roughened colony	Uniseriate spherical	Aspergillus sp
Deeply cotton, white Colony	Slightly Elongated	Rhizopus spp
Green rounded with White colony	Coarsely roughened	Candida spp
White to cream Coloured colony	Flat with Aerial mycelium	Geotrichum spp

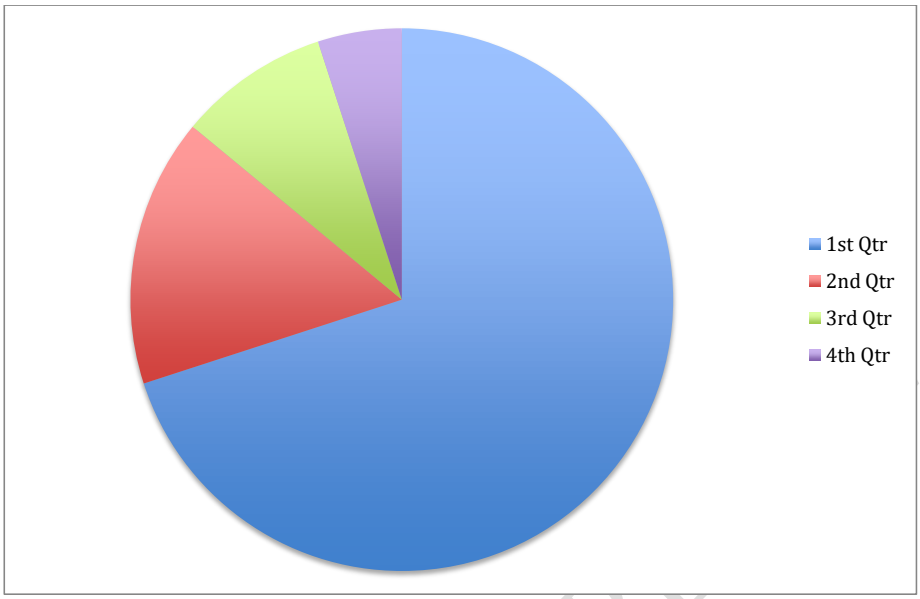

Figure 1: Percentage of occurrence of fungal species in sample (Water and Swab)

The results for the percentage of occurrence the fungal species in sample 1 is shown in figure 1 above. The figure shows that *Candida spp* recorded the highest percentage (70%), *Aspergillus* sp (16%) *Geotrichum* sp *(9%)* and *Rhizopus* sp (5%). The identification of the fungal species associated with the Jerry can of water vendors in sample 2 is shown below. The fungal species were identified based on their microscopic and macroscopic features. Three different fungal generas were identified; *Candida spp, Aspergillus sp and Fusarium spp*.

The results for the percentage of occurrence of the fungal species in sample 2 are shown in figure 2 above. The figure shows *Candida* spp recorded the highest percentage (66%) *Aspergillus* sp (19%) and *Fusarium* spp (15%).

The identification of the fungal species associated with the Jerry can of water vendors in sample 2 is shown below. The fungal species were identified based on their microscopic and macroscopic features. Three different fungal generas were identified; *Aspergillus sp, Rhizopus spp* and *Candida spp*.

Table 4: Fungal isolates from Sample 2 (Water and Swab)

Macroscopic Features	Microscopic Features	Fungi Isolates
Green rounded with White colony	Coarsely Roughened	Candida spp
Colourless, finely Roughened colony	Uniseriate spherical	Aspergillus spp
Pink colonies	Flat with Aerial mycelium	Fusarium spp

The result for the percentage of occurrence of the fungal species in sample 2 is shown in figure 3 above. The figure shows *Candida* spp recorded the highest (68%) *Aspergillus* sp (22%) and *Rhizopus* spp (10%).

The identification of the fungal species associated with the Jerry can of water vendors in sample 2 is shown below. The fungal species were identified based on their macroscopic and microscopic features. Three different fungal genera were identified namely; Aspergillus sp, Candida spp, and Rhizopus spp.

Table 5: Fungal isolates from Sample 3 (Water and Swab)

Macroscopic Features	Microscopic Features	Fungi Isolates
Colourless, finely Roughened colony	Uniseriate spherical	Aspergillus sp
Deeply cotton, white Colony	Slightly Elongated	Rhizopus spp
Green rounded with White colony	Coarsely roughened	Candida spp

The result for the percentage of occurrence of the fungal species in sample 3 is shown in figure 3 above. The figure again shows Candida spp recorded the highest percentage (58%), Aspergillus sp (25%) and Rhizopus spp (17%).

Discussion

This study was undertaken to assess the population of yeast and filamentous fungi associated with the Jerry can used by watser vendors (Meruwa) in Toru – Orua. The results obtained in this study showed different degrees of fungal contamination of the Jerry can of water vendors in Toru – Orua. The mycological analysis of the samples was done using culture dependent techniques. Table 1. shows the results for the fungal population in Jerry can swab and water samples cultivated on sabouraud dextrose agar. The results showed that the mean fungal counts of the swab samples ranged from 1.1×10^3 to 5.6×10^3 while the water samples collected from the Jerry can ranged from 0.8×10^3 to 1.2×10^3. The results suggest varying degrees of fungal contamination.

The cultivation and enumeration of the fungal species was also done on Yeast Extract Agar. The result obtained showed the mean fungal counts to be lower than those recorded by the Sabouraud dextrose agar. The mean counts obtained from the swab samples ranged from 1 to 20 while the mean counts for the water samples collected from the jerry cans ranged from 3 to 9.

Kirk et al. (2001) reported that yeasts and filamentous fungi have long been implicated as contaminants of drinking water. These organisms are prevalent in aquatic environments and have wide distribution because of their diverse metabolic capabilities. Thus, they have been reported to survive in low nutrient environments such as in the Jerry can of water vendors (Boguslawska – Was and Dabrowski 2001). The contamination of the Jerry cans may arise from the colonization of drinking water distribution system by yeast and filamentous fungi (Wurzbacher et al, 2011).

Another factor that may influence the prevalence of yeast and filamentous fungi in Jerry cans is the formation of biofilms. Biofilms are an important habitat for fungi in drinking water (Wurzbacher et al, 2011), it suggested that the problem of biofilm formation is common in Jerry cans because it provides a favorable environment for their growth and survival. Biofilms also promote the growth of filamentous fungi and yeasts, the formation of biofilms can serve as a source of nutrient and home to other harmful microorganisms, thereby putting hundreds to thousands of consumers at great risk. Yeast and filamentous fungi have also been reported by several studies to survive after filtration (Amaurya et al., 2005).

Different species of fungi were identified in this study. From the swab samples in location 1, *Aspergillus* sp., *Rhizopus* spp., *Candida* spp., and *Geotrichum* spp. These fungal isolates recorded different prevalent rates. *Candida* spp recorded 70% of occurrence, *Aspergillus* sp (16%), *Geotrichum* spp (9%) and Rhizopus (5%). In location 2 swab samples, three (3) different fungi genera were identified; *Candida* spp (66%), *Aspergillus* sp (19%) and *Rhizopus* (15%). In location 3 water samples obtained from Jerry cans of water vendors, three (3) fungal genera were identified; *Candida* sp (68%), *Aspergillus* sp (22%) and *Rhizopus* sp. (10%). The occurrence of opportunistic yeast and filamentous fungi in water containers suggest a potential risk to direct water users because some of these toxins produced by fungi pose risks to humans and animals.

The fungal genera isolated in this study are similar to the findings of other related studies. In the work of Amaurya et al., (2005), a wide variety of fungal genera were isolated from water. Some of these (*Penicillium*, *Trichoderma* and *Aspergillus*) and are known to be strongly allergenic and can induce skin irritation, or may cause infections in immunosuppressed individuals (Amaurya et al., 2005).

In other studies by different researchers (Alangadan, 2011; Enoch et al., 2006; Walsh and Groll, 2000), it was reported that the most commonly isolated drinking water fungi, *Aspergillus* spp and *Fusarium* spp. have been recognized

as prevalent opportunistic pathogens. Previous research had recovered diverse fungal species from drinking water, and the spectrum of waterborne fungi contain multiple opportunistic fungal pathogens (*Aspergillus* spp., *Fusarium* spp., *Acremonium* spp., and *Trichoderma* spp). As previously suggested, drinking water has the potential to beone route of transmission for these opportunistic fungal pathogens (Anaossie et al., 2001).

According to Siqueira et al (2011), the filamentous fungi from different genera (*Aspergillus, Fusarium, Acremonium Alternaria, penicillium, Mucor and Rhizopus)* have often been detected in tap water. The presence of specific species of filamentous fungi and yeast in Jerry cans of water vendors directly indicates a poor sanitary state and hence an epidemiological threat.

Conclusion

This study was aimed at assessing the prevalence of yeasts and filamentous fungi associated with the Jerry cans used by water vendors (Meruwa) in Toru – Orua. Yeasts and filamentous fungi contaminate the Jerry cans of water vendors are contaminated by yeasts and filamentous fungi. *Candida* species was observed to be the most dominant yeast species associated with the Jerry cans while *Aspergillus* spp, was the most dominant in the water samples collected from the Jerry cans. The fungal genera identified in this study includes; *Candida* spp, *Aspergillus* spp, *Fusarium* spp, *Geotrichum* spp and *Rhizopus* spp. the presence of these fungal species may pose health risk to consumers of the water drawn from the contaminated Jerry cans.

5.2 Recommendations

From the data generated from this study, the following recommendations were made;

1. The prevalence of yeast and filamentous fungi in the Jerry cans used by water vendors may result to illnesses. Thus, Jerry cans should be properly and regularly washed and disinfected to reduce the risks of fungal infections arising from the consumption of fungal contaminated water.

2. Various factors influencing the prevalence of yeasts and filamentous fungi in Jerry cans used by water vendors. Therefore, more studies should be conducted to evaluate the influence of the environmental factors on the prevalence of yeasts and filamentous fungal species associated with the Jerry cans.

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Comment [T2]: References listed in the order that they appear in the text.

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